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THE SUBSTANCES NEEDED FOR THE GROWTH OF A PURE CULTURE OF *COLPIDIUM COLPODA*.

By R. A. PETERS, M.D., *Fellow and Lecturer of Gonville and Caius College, Cambridge.*

(*From the Biochemical Laboratory, Cambridge.*)

THOUGH considerable attention has been given to the nutritional needs of bacteria, the protozoa have largely escaped notice. This seems to be mainly for the reason that it has been generally believed that they required complicated chemical bodies for growth, if not actually bacteria. During the course of some other work, a pure culture of protozoa was wanted. Reference to previous work did not elicit any information upon the means of obtaining such a culture, free from bacterial and other organisms. Some work, however, by Hargitt and Fry(1), shows how paramœcia may be kept alive upon definite strains of bacteria, after freeing from other organisms.

The following paper is an account of the means by which a strain of *Colpidium colpoda*, reared from one individual, has been kept alive for about ten months, free so far as can be ascertained from other organisms. The minimum needs of this strain in inorganic and organic substances have been sketched as a preliminary to work upon a larger scale.

The ciliate organism finally used belonged to the variety usually known as *Colpidium colpoda* (Ehrbrg) Stein[1]. It was obtained from a hay infusion. It has a mean length of about 50 μ, and has one micronucleus (cp. Fig. 1).

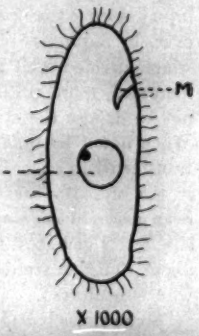

Fig. 1. *Colpidium colpoda* (Ehrbrg) Stein. × 1000. Specimen fixed in osmic acid. *N* = macronucleus, one micronucleus inside. *M* = mouth.

Methods. In a preliminary communication(2)[2] the medium has been described in which the original isolations were made. The method of starting the cultures was to a large extent

[1] I am indebted to Mr Clifford Dobell, F.R.S., for kindly identifying the organism for me and for some helpful criticism.

[2] The organism was here erroneously described as paramœcium.

that used by Hargitt and Fry. A Colpidium was isolated from a hay infusion culture, in a drop of sterile medium upon a sterile microscope slide. A capillary pipette made of drawn-out glass tubing and fitted with a rubber teat was used both for this operation, and for the subsequent transfer of the organisms. The protozoa were kept under observation during these operations by watching the results with a stereoscopic binocular microscope. The isolated Colpidium was washed through several changes of sterile culture medium upon sterile microscope slides. Between each washing the pipette was carefully heated in order to keep it sterile. (The operations should be performed in a room with as little draught as possible, to avoid the chance infection of the drops upon the microscope slide. The quicker the operation can be made, the greater the chance of success.) After about six washings, the organism was transferred to a fresh drop of sterile medium, in a two inch depression block covered with a small sterile cover-glass, both of which had been previously sterilised by heat. A trace of vaseline was usually placed between the block and the cover-glass to prevent evaporation of the drop. The process is rather tedious as there is always the chance of injury during the operations as well as other disturbing factors. However, by a process of trial and error, organisms were finally obtained which divided well in the sterile fluid. As the number of colpidia increased, more culture medium was added to the block, taking care not to expose the sterile culture to the air more than was necessary. When about 20 organisms had been obtained, the whole culture was transferred to a small tube, previously sterilised and fitted with a plug in the ordinary bacteriological way. After a good active culture had been grown in this way, it was found possible to subculture into ordinary size, 6 × ⅝ inch, test-tubes fitted with cotton wool plugs and containing about 10 c.c. of sterile medium. One or more drops of protozoal culture were subcultured.

Some stress is laid upon the relation of the volume of the medium to the number of colpidia growing in it at these early stages, as it is believed that this is an important factor. Many attempts have been made to grow individuals isolated in small test-tubes in about 1 c.c. of medium, all of which have been failures. It is suggested that in order that growth should proceed, the organism has to modify its environment, probably by adding to it some synthesised substance in sufficient concentration for growth. The matter must be closely related to the so called "bios" effect with yeast. When a sterile medium containing 20–40 organisms has been obtained, the subsequent subcultures are easily made.

Out of several strengths of the medium the following one, Table I, gave a successful culture.

TABLE I.

	p.c.		p.c.
Sodium chloride	·06	Glucose	·03
Potassium chloride	·0014	Histidine	·01
Calcium chloride	·0012	Arginine	·01
Basic sodium phosphate (Na_2HPO_4)	·0001	Leucine	·01
Acid potassium phosphate (KH_2PO_4)	·0001	Ammonium lactate	·003
Magnesium sulphate	·001	Ferric chloride ...	Trace
Sodium bicarbonate	·002	Potassium iodide ...	Trace
Phenol red	Trace	Manganous chloride	Trace

The substances were made up with glass-distilled water. The constituents were autoclaved separately, and the final mixture sterilised by heating to 80° C. on three successive days. The reaction was adjusted (3) to $p.H = 7{\cdot}4 \pm 0{\cdot}4$ with NaOH N/100. The indicator, phenol red, was added to show any changes in reaction occurring during growth. It has a convenient range round about the neutral point and does not affect the protozoa.

Preliminary trials in hay infusions of various reactions showed that the organism grew best when the reaction was initially $p.H = 7{\cdot}0 - 7{\cdot}4$, about the reaction of human blood, though variations of 0·6 did not seem to make much difference. D. Dale (3) has shown that the limiting acid and alkaline reaction for the growth of *Paramœcium caudatum* is between $p.H$ 5·0 and 9·0.

In the later work a silica pipette was used in place of glass, made of drawn-out ¼-inch silica tubing. It had the great advantage of standing heat and does not contribute doubtful substances to the media. In all cases, unless stated, glass distilled water was used. In the earlier experiments this was obtained by distilling tap-water to which baryta had been added. In the later experiments where it was desired to eliminate the possibility of organic nitrogen, tap-water was distilled from alkaline potassium permanganate, and the middle portion of water coming over used. The method of obtaining the special water used in the potassium research is described below. The chemicals were Kahlbaum's practically throughout. In the experiments upon the inorganic constituents, Kahlbaum's "Zur Analyse" salts have been used.

The successful culture was tested for bacteria in the ordinary bacteriological way. Drops from the growing culture were transferred with a platinum loop to the following four media, 1 nutrient broth, 2 nutrient agar, 3 glucose agar (anærobic) and 4 litmus milk.

Preparation of media. 1. Nutrient broth. 1 pound of bullock's heart was minced and 1 litre of tap water added. The mixture simmered for one hour, was boiled for 30 minutes,

filtered, neutralised, with N soda. Peptone was then added to a strength of 1 p.c. and NaCl to ·5 p.c. It was then heated, neutralised with N soda to $p.H = 7{\cdot}4$ and heated, filtered, put up into test-tubes and sterilised on three successive days at 100° C.

2. Nutrient agar. The above + 2 p.c. agar added.
3. Glucose agar 2 + 1 p.c. glucose (aerobic).
4. Litmus milk. Separated milk + neutral litmus.

Subcultures upon 1 and 2 were left in the cold (15° C.) for two days, when they showed no sign of bacterial growth. They were then incubated at 37° C. and no growth was obtained in a fortnight. On the second day the broth cultures were subcultured upon 2, and put in the incubator at 37° C. showing no growth after a week.

No growth could be obtained with media 3 (anaerobic) and 4 in the cold or heat. Control cultures on these media in cold and heat, made from the original hay infusion from which the paramœcium was isolated, showed a good growth in 16 hours.

After the failure of growth on the ordinary culture media, some slant tubes of media made from the amino-acid culture medium stiffened with agar were set up. Subcultures upon these from a growing culture produced no result.

A further control experiment was done in the following way. Colpidia will pass through one filter paper, but not through two. A filter funnel containing two filter papers was sterilised. A growing culture of colpidia was then filtered through the sterilised filter papers into a sterile test-tube. The filtered medium contained no visible colpidia. About 1 c.c. of the filtered medium was put into a fresh tube containing 10 c.c. of the same medium. This tube was fitted with a cotton-wool plug, and covered loosely with tinfoil to avoid the entry of dust. (Previous experiment has shown that colpidium will grow under similar conditions.) After a month no change could be detected in the medium. Seeing that the filter papers used were large enough to pass colpidia in one layer and would also let through *B. subtilis* cultures in two layers it seems unlikely that any other organism would not come through. This therefore indicates that there are no other organisms present which can be cultured apart from the colpidia. Microscopic examination using the ordinary bacteriological stains failed to show any bacteria, even after crushing the protozoa.

We were, however, puzzled for a time over the following fact. In certain cases (especially at the beginning of the work) an examination of the living culture in a hanging drop preparation under the 1/12 oil immersion lens showed the presence of rod-shaped, apparently motile bodies. Upon cursory examination they rather resembled bacteria. When watched carefully, however, it could be seen that their movements were restricted at one end, they appeared to be fixed to the cover slip. Often they occurred in groups. They were never observed to divide. They could not be fixed by heat. When fixed by osmic or iodine vapour in

chloroform, they were difficult to stain. It was suggested in the preliminary account that they were knocked off cilia, and this explanation has received confirmation.

Firstly. After subculture in the ammonium glycerophosphate medium for four months, an examination of the living cultures showed none of these bodies. This was not understood until a chance experiment upon a phosphate deficient medium gave a clue to the matter. In this medium, on the seventh day after sowing, it was noticed that the protozoa were apparently rounding off and forming cyst-like bodies. An examination of the culture in the hanging drop preparation showed the presence of many of the rod-shaped bodies, which were not present in the control culture. This seemed confirmatory evidence of protozoal origin.

Secondly. It was found that if a hanging drop preparation from a colpidium culture containing none of these bodies was made in a chamber through which CO_2 could be passed, and if CO_2 was passed through the chamber while the preparation was observed under the 1/12, as the protozoa disintegrated, these bodies appeared. This disintegration in some cases leads to the splitting off of patches of epithelium with cilia attached to them. The direct observation of disintegration seems to afford the strongest proof that the bodies observed arose from the protozoa themselves. The fact that the disintegrating action of CO_2 produces these bodies so quickly seems to put out of court a possibility that they might be symbiotic or parasitic organisms, similar to those described by Petenschko(4) and others for paramœcia. The possibility that they are some hitherto unknown form of colpidium itself seems unlikely. For if they were, growth of colpidia might well have been expected in the filtered culture.

The weight of the evidence is that the cultures really contain no other organisms than the one in question. This is to a certain extent confirmed by the appearance of chance infections during the course of the work. At times cultures have been found to contain bacteria when tested upon nutrient agar. They are in most cases easily picked out by the naked eye as they show the cloudiness usually seen in a bacterial culture. Cultures of colpidia generally look fairly clear unless they contain exceptional numbers of the organism (10,000 or more per c.c.).

It must be confessed that it has been a surprise to find that it was possible in an ordinary laboratory to keep a strain of protozoa sterile for so many months in spite of the many subcultures made. The technique has been carried out carefully with bacteriological methods, but even then it would seem that there must have been some other element at

work—either the killing of small chance infections by the colpidia themselves, or the fact that the media used were not on the whole suitable for the growth of organisms like *B. subtilis*. This was certainly true for the amino-acid media. A sudden clouding of the medium with bacteria is usually followed by the disappearance of the protozoa. This type of effect has been observed by others. Thornton and Smith(5) found that they obtained the best growth of Euglena by sowing into a culture medium in which the bacteria could not multiply fast. An attempt, however, to find out whether colpidia could eat up bacteria when added to the culture was not successful. The bacteria were still present at the end of some days and the protozoa were much diminished[1].

Nutrition. The growth of colpidium in culture media containing only simple inorganic and organic substances raises the interesting question as to the mode of nutrition in such cultures. Mr Dobell informs me that it is generally accepted that ciliates feed upon solid food. In these cultures, the question of nutrition could not be settled without experiment directed to the point. Ultimately it seems clear that the food material comes from simple substances in solution. Is it absorbed from the inside or the outside? A growing culture usually contains after some time a mass of debris, excreta and otherwise, from the colpidia. This may well serve as a source of nutrition, in the sense that substances in solution condensed in the particles may be swept into the organisms; but the very great growth in clear media (up to 20,000 per c.c. in some cases) would suggest that there is also some direct absorption. That they are not growing upon the bodies of killed bacteria in the distilled water from which the media was made up, is proved by the following considerations. First, if they were, selective growth on different carbon sources should not take place. Secondly, the distilled water was in most cases used directly, and experiments upon the ammonium glycerophosphate medium, in which it was filtered before sterilising did not give different results. Thirdly, distilled water put up in the ordinary way and kept for a fortnight when tested for bacteria gave no growth on planting out 1 c.c. portions upon plate culture media.

The cultures have been grown at laboratory temperature ranging from 15–24° C.

Change of reaction during growth. During the process of growth the

[1] Attempts to apply the same method of culture to *Paramaecium caudatum* have not yet been successful. Though this organism has been freed successfully from bacteria, it has not been possible to free it from a small flagellate. Whether this is a case of symbiosis or parasitism has not yet been determined.

reaction in most cases changed. A medium containing ·06 p.c. NaCl and ·06 p.c. ammonium glycerophosphate for instance goes acid during growth. On the other hand if the medium contains ·03 p.c. NaCl and ·03 p.c. NH_4Cl instead of ·06 p.c. NaCl, the reaction goes alkaline during growth. In the former medium the protozoa tend to bunch at the bottom of the culture tube and in the latter at the top. This must be in some way an attempt to get to the neutral zone because, if a tube of the latter medium is inoculated and not disturbed, and the initial reaction is made slightly acid, the top of the medium will turn pink before the lower part changes. (Phenol red is yellow when acid and pink when alkaline.) The colpidia will be seen in the greatest numbers at the point where the pink joins the red fluid. The orientation of these protozoa to hydrogen ion concentration seems to show that they are not so markedly affected by oxygen concentration, as has been observed to be the case for certain other flagellate organisms.

Methods of estimating growth. It was found at a comparatively early stage that the following medium (NaCl ·06 p.c., KCl ·001 p.c., $CaCl_2$ ·002 p.c., $MgSO_4$ ·001 p.c., ammonium glycerophosphate 0·06 p.c., phenol red trace) made with glass distilled water and put up in amounts of 10 c.c. into test tubes would suffice for the needs of growth. In this medium ammonium glycerophosphate serves as the nitrogen, carbon and phosphorus source. It will be known throughout as the "ammonium glycerophosphate medium." The strain of colpidia have been kept going upon it for a period of four months by frequent subculture, so that it apparently contains all the essential constituents for growth. The colpidia in each subculture do not get appreciably smaller as judged by the length at the time of maximum growth. If a test-tube containing 10 c.c. of this medium is inoculated with a drop from a sterile culture, growth will take place in the course of two or three days. The growth will vary a bit with the temperature tending to be rather faster at 23° C. than at 18° C.

In order to get comparative results, it was necessary to obtain a technique for estimating the growth. Two methods were used throughout the research.

1. *Approximate method.* An approximate idea of the growth was obtained by shaking the culture tube and placing it in a parallel-sided glass trough almost the same size as the test-tube. The trough was then filled with water and the contents of the test-tube examined with a binocular microscope, using a 1-inch objective. With some practice a rough judgment of the growth can

be marked out of 10, where 10 represents the maximum growth possible. The results are taken by one observer and marked down by the other so that in this way an independent judgment is got. The daily results are then charted. Fig. 2 shows such a chart, upon the ammonium glycerophosphate medium. The ordinates represent growth 10 divisions = 10 (maximum growth). The abscissæ represent days. The vertical arrows represent the making of a new subculture. It was found that the organisms did not subculture certainly unless there were 20 or more transferred in the drop to the new culture tube of 10 c.c. Cultures marked as 2 or more by judgment were usually good enough to subculture.

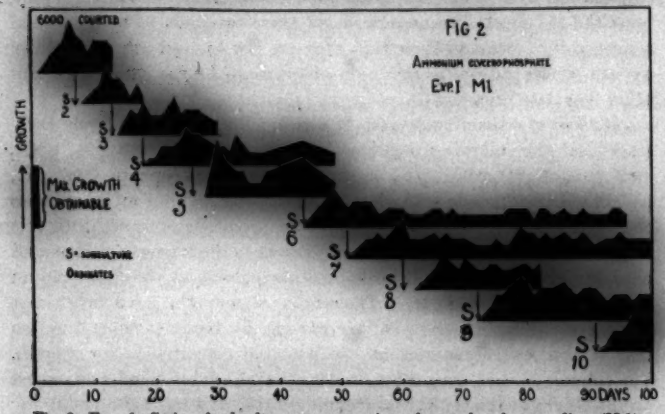

Fig. 2. Exp. 1. Series of subcultures on ammonium glycerophosphate medium (M 1).

Those marked 1, and it was the practice to mark them "one" if they showed life, did not always subculture.

There are two main sources of error, (1) the personal equation and (2) the fact that the colpidia sometimes bunch together and so are apt to give a false idea of the number present. In many cases, if this was expected, a count by method 2 was also made. A mark of two and under was usually under 2000 per c.c. when checked by counting. A mark of three to about six tended to increase from 2000 to 6000 or 7000 per c.c. Above this 10 meant 10,000 or over per c.c. The maximum ever observed by counting has been 40,000 per c.c.

In spite of its roughness, the method is quite good enough to judge of the time for subculturing. Further it has the great advantage of

speed, and of avoiding any extra risks of contaminating the culture liable to occur if the tube is opened and some of the fluid withdrawn.

Fig. 2 shows the following things. For any given subculture there is a rise in growth to a maximum, and then a gradual fall to what is apparently a constant condition. The colpidia are gradually dwindling in this period in size. A few organisms will survive for a long time in one of these sterile cultures. They have been observed after a period of 6 months. In order to analyse what was going on more carefully, about 100 c.c. of the medium was put up in 200 c.c. flasks fitted with cotton wool plugs, and the organisms were counted regularly by the method described below.

In many of the figures showing the daily growth as judged by the ocular method, the culture appears to die down temporarily and then recover. This is especially noticeable in the media which were deficient in some important constituent (*e.g.* M 31, 2, Fig. 10). The result is not purely artificial, as is shown by daily counts of flask cultures, where very large variations in the numbers may occur from day to day. It would seem that growth takes place up to a certain limit, then there is some kind of failure. Some organisms survive and are able to use the disintegration products of the others. This is supported to a large extent by the results of the deficient medium M 31, for instance.

There may also be the additional factor of cyst formation contributing to this result. Cysts have often been observed on the walls of the test-tubes during the course of the work.

2. *Accurate method.* The test-tube was shaken, the plug carefully withdrawn and some of the culture removed with a sterile silica pipette. Holding the pipette vertical two drops were allowed to fall respectively into two separate drops of strong iodine in potassium iodide previously placed upon the microscope slide. The iodide both fixes and stains the protozoa, which can then be easily counted under a 1-inch or Zeiss *A* objective. Successive counts in most cases show surprisingly good agreement. The volume of the average drop delivered by the pipette in a vertical position can be found by weighing, and a simple multiplication gives the number of organisms per c.c. of the culture. Duplicate counts do not as a rule differ by more than 10 p.c. At the same time that the counts are made, measurements of the length can be made with a micrometer eyepiece. This should be done immediately before counting, as the organisms are apt to shrink. The average length was taken by estimating the length of 10 organisms taken at random. It has been shown by other observers that length gives the best idea of the size of the organisms. Broad organisms usually mean that division will soon take place.

The course run by a growing culture, Exp. 2, ammonium glycerophosphate medium, upon which counts have been made regularly, is

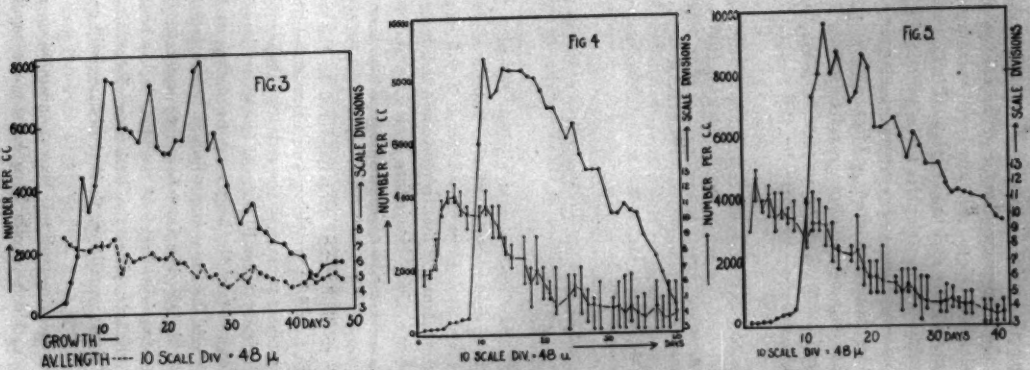

Fig. 3. Growth curve of colpidium culture upon ammonium glycerophosphate (M 1).

Fig. 4. Growth of colpidium culture on Medium 2. { Maximum length. Average length. Minimum length.

Fig. 5. Growth of colpidium culture on Medium 2.

shown in Fig. 3. This flask was studied over a period of 48 days. It will be seen that, broadly speaking, the growth can be divided into three periods: (1) 1–12 days inclusive, (2) 13–28 days and (3) 29 days to the end. The first phase shows a gradual increase in the number of organisms up to a maximum of 7500 per c.c. During this time the length kept between 7·2 and 7·9 scale div. During the second phase the number of organisms tends to fluctuate between 5100 and 8000, with a gradual and variable diminution in the length. During the third phase the number of organisms gradually fell until it remained constant at about 1500

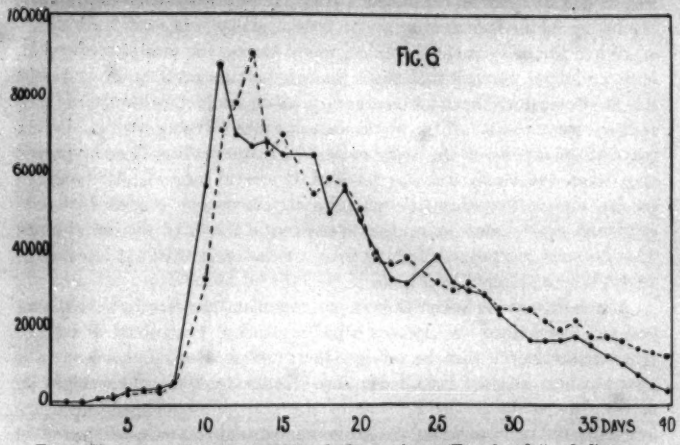

Fig. 6. Variation in living material in colpidium cultures (Exp. 3 and 4). Ordinates = product (no. per c.c. × mean length).
—— Exp. , Fig. 4. ---- Exp. , Fig. 5.

per c.c. while the length averaged about 5 S.D. The extreme variations found in the lengths of organisms tended to be more in the later stages.

In order to find out what variations were likely to appear between two cultures treated in the same way, two parallel experiments were done with a medium in which a substitution of ·03 p.c. AmCl, ·01 p.c. ammonium phosphate, ·02 p.c. glucose, ·02 p.c. sodium maleate, and ·01 p.c. leucine was made for the ·06 p.c. ammonium glycerophosphate of the ammonium glycerophosphate medium. Figs. 4 and 5 show the results. The curves are very similar to Fig. 3. Fig. 5 lags behind Fig. 4 by about a day, probably due to a slight difference in the number of

organisms originally sown. In Fig. 6 the values (number per c.c. × average length) for Figs. 4 and 5 have been plotted to obtain an idea as to the average content of living material from day to day. It will be seen that the curves for this product follow one another quite closely. They reach a maximum on the 10th–12th day after which there is a gradual fall. An inhibiting factor appears upon the 12th day, which may be due to many causes. It might be due to lack of food material (the most likely cause), or to the accumulation of toxic material in the medium, or to a balance being established between the number of organisms growing or the number of organisms dying. In a large 5-litre culture the inhibiting factor appeared at about 2000 organisms per c.c.

When the subsequent or middle phase starts, the numbers of organisms no longer increase and there is a gradual decrease in size. As the size is decreasing, there are temporary increases in the number of colpidia present per c.c. This would seem to prove that growth is taking place at the expense of the living material already present. For supposing that when the maximum was reached, the organisms merely dwindled in size due to inanition, it would be expected that the curve would gradually come down owing to the accidental death of the individuals. This does not happen. After dropping it rises again, without increase in protoplasm as judged by Fig. 6.

Hence it may be taken that in the organism increase in protoplasm and cell division are two factors capable of being dissociated. Dr E. C. Grey has told me that he noticed the multiplication of bacteria in a poor medium, as they dwindled in size. He thought that there might be division going on at the expense of the protoplasm. These colpidium curves would seem to prove it. A phenomenon of this sort is apparently common in the dividing ovum. Dr Shearer tells me that the first divisions take place without increase in size of the whole mass. The diminution in size due to lack of food material has been observed by many. The most elaborate studies seem to have been made by Jennings(6) and Wallengren(7) on paramœcia. Jennings has shown that diminution in size may be due to several factors among which the most important in this connection is the food material, other causes are of course mainly connected with heredity.

That in the cases of these sterile cultures, there was no permanent change in the size of the organism was readily shown by the following experiment. Colpidia were taken from three old cultures, in which the paramœcia were seen to be small with the microscope. The average length for ten organisms was taken. Subcultures were then made into

three fresh tubes of the same medium. After 5 days the average length of the organisms in the new cultures were taken. Table II shows the result.

TABLE II.

Culture	Length in old culture	Length in new culture
A	3·9 S.D.	8·5 S.D.
B	5·3	10·0
C	5·3	9·0

10 scale divisions (S.D.) = 48μ.

The minimum needs of Colpidium colpoda. With the present technique, it has not been possible to do more than outline the substances which must be added to the water to get growth. Traces of other substances were probably present throughout the research both in the water and in the pure salts employed. This does not really affect the question here, as the object is to find out what substances must be added. If growth will not proceed under the conditions of the experiments without the addition of a given substance, that substance may be considered to be essential. Taking the ammonium glycerophosphate medium as a standard, each of the constituents has been omitted in turn, replacing the missing constituent if necessary with some other substance to adjust the osmotic pressure. It has been found that small changes of osmotic pressure (as represented by the range, ·06 p.c.–·12 p.c. NaCl) are compensated for by the organism. Owing to the time required it has been impossible to do daily counts of all the cultures. In most cases charts of the growth have been made by the ocular method. The real criterion is whether successful subcultures can be made, because the first and probably the second culture upon a new medium will still contain traces of the missing constituent from the original medium. The character of the growth as compared with the control, and whether it was found necessary to resow or not (marked *R* upon the charts) also gives valuable information. In some cases, *viz.* potassium and carbon compounds, the effect of certain substitutions have been tried.

The technique followed was in practically all cases the same as that described for the medium in Table I, 10 p.c., 1 p.c., or 0·1 p.c. strengths of the constituents being made up as was most convenient. It was usually found convenient to make up 50 c.c. of a new medium. From this, four 10 c.c. amounts were transferred by pipette to cleaned and sterilised test-tubes. The test-tubes were cleaned with soda, and nitric acid, washed out with ordinary distilled water and then with several changes of the water used for the experiments. They were then dried, excluding dust as far as possible, fitted with cotton wool plugs and

sterilised at 150° C. for an hour. The test-tubes containing the medium were sterilised in the ordinary way, by steaming on three successive days.

In cases where a batch of media varying in only one or two constituents had to be made up, these latter were made up as a basal mixture ten times the strength of the final medium, to secure uniformity of the constant parts of the medium. 5 c.c. of the basal mixture were then transferred to a 50 c.c. flask, the extra constituents added and the whole made up to the mark with the necessary water.

The four tubes of any new medium were labelled 1, 2, 3, 4, and stored in a dust proof way for use. A subculture was made from a standard or other medium by transferring a drop from the standard tube to tube 1 of the new medium in the ordinary bacteriological way. When the first culture reached a mark of three by inspection, tube 2 was sown from tube 1 and so on with tubes 3 and 4. In the second tube the percentage of the original medium for a drop of 1/25 c.c. would be 0·04 p.c. Anything added to the medium by the colpidia in the course of their growth is not eliminated by this method. This could only be done by washing several times with the centrifuge, a proceeding which would largely increase the chance of infection and disturbance of conditions.

The inorganic basal diet. The following ions had to be investigated, anions Cl, SO_4, PO_4, kations NH_4, Mg, Na, K, Ca.

In the earlier experiments, the mixture of $FeCl_3$, $MnCl_2$, and iodide was added (called iron mixture). Later this was omitted when it was found that the organism could be taken through six subcultures in a medium to which this mixture had not been added. As it has been pointed out above, the fact that these substances did not have to be added does not say that they are not essential to the manifestation of life. Traces of them may quite well have been present in the pure salts used in the work. To obtain cultures it was not necessary to add them specifically. Fig. 7 shows the results of chloride, sulphate, phosphate and ammonium deficiency in duro glass test-tube cultures, together with a control culture (M 3) on the ammonium glycerophosphate medium containing the iron mixture.

Chlorides. M 3, the control grew well and subcultured. In M 4 the chlorides were reduced to the small amounts present in the iron mixture by substituting for $CaCl_2$ and KCl, Na bicarbonate ·02 p.c., KH_2PO_4 ·002 p.c., $CaCO_3$ ·001 p.c., and raising the concentration of ammonium glycerophosphate to ·10 p.c. Growth was not good, in fact the last subculture hardly grew. In M 5, the traces of chloride in the iron mixture were removed by leaving out the iron mixture from M 4. A subculture

could not be obtained upon resowing. That this is not due to any toxic effect of the new medium seems apparent from the fact that when the first subculture was made growth was obtained.

Phosphate. M 6 and M 7, in which ammonium glycerate, ·08 p.c., was substituted for ammonium glycerophosphate, show the effect of leaving out phosphate, M 6 contained the iron mixture, M 7 did not. M 6 did not grow at all in the first two tubes even after a resowing. In the third tube growth was obtained, though the fourth tube, sown from tube 3, would only grow upon resowing, suggesting the carrying over of

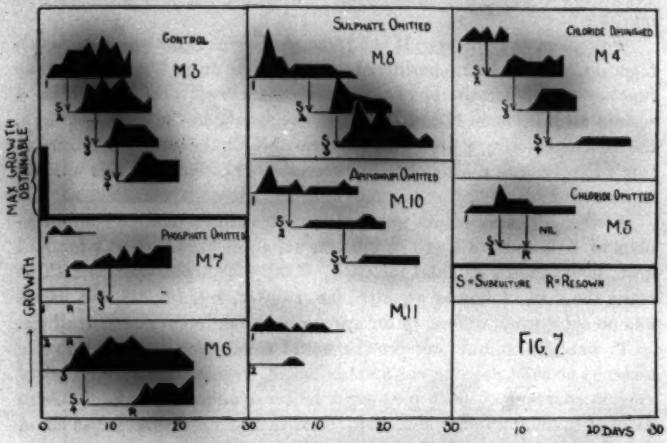

Fig. 7. Effect of deficiency of the medium in chloride, sulphate, phosphate and ammonium. Scale same throughout.

traces of phosphorus from tube 3 to tube 4. In M 7 no growth was obtained in tube 1, though the organisms lived a day or two. A second tube sown grew, but gave no subculture. The contrast with the control is striking. In some later experiments it was found that lack of phosphate led to apparent disintegration of the organisms.

Sulphates. M 8 and M 9, in which $MgCl_2$ was substituted for $MgSO_4$, show the effect of leaving out sulphates. M 8 contained iron mixture, M 9 did not. On the whole the organism seems to get along without any added sulphate. This experiment does not prove whether sulphates are necessary for growth or not, as it is not possible in laboratory conditions

at the moment to be sure that minute traces of sulphur are really excluded. Further experiments with M 9 in quartz tubes made up with the special water described later elicited the same result. There is no doubt, however, that sulphur has been reduced to a very small quantity, without interfering substantially with growth. Other observers (see Czapek) have observed a similar result for certain bacteria.

Ammonium. M 10 and M 11, in which sodium glycerophosphate was substituted for ammonium glycerophosphate, shows the effect of leaving out ammonium. As would be expected growth will not take place. The ammonium is the nitrogen source. (M 11 contained no iron mixture.)

Potassium and calcium. It is convenient to consider these two metals together. It has been considered somewhat as an axiom that for the functioning of certain physiological mechanisms, there should be a certain ratio between K and Ca. At the beginning of the work therefore, the solutions were prepared with the ratio of K/Ca the same as in ordinary frog's Ringer. The results of later experiments showed that this was not necessary.

A series of experiments were made with various relative concentrations of KCl and $CaCl_2$ (40 in all), the range of variation was for KCl from ·00037–·0026 p.c. and for $CaCl_2$ ·00062–·0022 p.c. Between these limits the ratios followed were 0·6, 0·8, 1·0, 1·2, 1·4, 1·6, 1·8, 2·0. There was no substantial difference for growth in the different concentrations.

It was then found that growth would take place quite well in the presence of solid calcium carbonate. Again, keeping the percentage of calcium constant at ·0013 p.c., extra KCl was added to make the ratio $KCl/CaCl_2$, 2·75, 3·5, 5·0, 6·6, 12·0. Growth was normal so far as could be judged though rather slower in starting in the higher concentrations, probably due to an osmotic effect. It would appear therefore that for this organism the concentrations of KCl and $CaCl_2$ can be altered within wide limits. The changes in the medium compatible with growth, argue a wide independence of environment upon the part of this strain.

There followed next the question as to whether potassium and calcium were necessary for growth. In the case of the higher plants, these two elements are certainly essential, and this is evidently the case also for the algæ, and for molds and bacteria (8, 9).

Fig. 8 shows a series of four experiments (Media 12 to 19) in which growth on a control medium is compared with growth on the same medium with the potassium omitted. M 12, M 14, M 16, M 18 were the ammonium glycerophosphate medium without the KCl. M 12–M 15

inclusive contained iron mixture, M 16–M 19 did not. Phenol red was omitted in these and subsequent experiments on potassium, uranium,

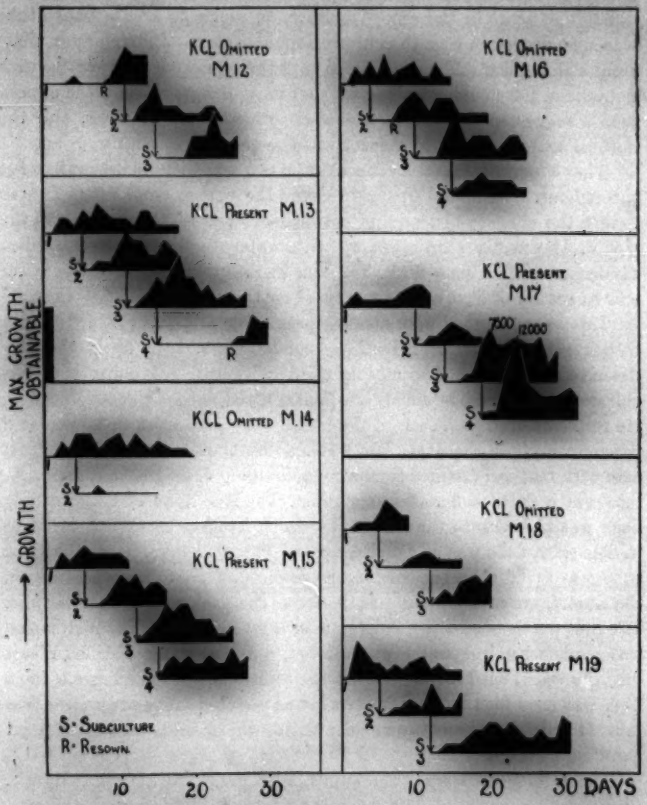

Fig. 8. Effects of potassium deficiency in glass test-tube cultures. Description as Fig. 7.

calcium and sodium. The experiments were made in duro-glass test-tubes. The controls to the series were M 13, M 15, M 17, M 19. It will be seen that the results are somewhat variable. For instance M 13 sub-

cultured well, M 12 subcultured after a resowing. M 15 subcultured, whereas its fellow without potassium would not subculture. M 17 subcultured very well, M 16 not so well. M 19 subcultured well, M 18 was difficult to start in the second culture, so much so that another tube was subcultured in case the culture should be lost. Judging from these results alone, it would be concluded that the organisms grow better with potassium, but could get along without it. The variability of the experiments suggested that there was some other factor at work, and the natural source of error to suspect was the glass.

The experiments were therefore repeated with silica apparatus throughout. Muslin was used to cover the cotton wool plugs and so reduce the chances of traces of cotton-wool finding their way into the media. The media were made up with water distilled through a silica condenser from a Jena flask. The aim in the preparation of the water was to get rid of dust and of metals other than ammonium.

Tap water, treated with alkaline permanganate, was distilled from a distilling flask fitted with a Liebig condenser, and set up in the way described by Grey[1]. The middle third of the water coming over was collected and stored in Jena glass flasks, fitted with corks covered with tin foil. The cork and neck of the flasks were covered with tin-foil and paper to exclude the dust. The water so obtained was then made just acid with HCl and distilled from a Jena distilling flask, 2000 c.c. capacity, into which fitted a Jena glass stopper. The side tube of the distilling flask was passed into the silica tube which had the condenser fitted to it. The silica tube was of $\frac{3}{4}$-inch bore and 30 inches long. Over the silica tube was fitted a Liebig condenser. A piece of tin-foil served to keep the dust from entering the join between the flask and the silica tube. The first portions of water coming over were neglected. The distillation was made when the water was wanted, and the water distilled direct into the vessel used for making up the solutions. In most cases a silica flask was used for this purpose. As far as possible every precaution was taken to prevent the entry of dust during the making and subsequent handling of the media.

The salts used were Kahlbaum's, first grade KCl, $CaCl_2$, $MgSO_4$. Phenol red was not used. The uranium acetate used later was a Kahlbaum sample. The ammonium glycerophosphate was a Kahlbaum sample, but not guaranteed. The solutions of KCl, $CaCl_2$, $MgSO_4$, were stored in silica tubes. The ammonium glycerophosphate was measured direct into

[1] I am able to take the opportunity of thanking Dr Grey for his valuable advice upon the preparation of the water.

the flasks by a drop method from the silica pipette. Any washings needed were done with some of the water stored in a silica flask.

When the experiments upon potassium deficiency were repeated in silica test-tubes, the differences were striking. Fig. 9 shows the results. The series M 21, 1, deficient in potassium, and the control; M 22, 1 and 2, were made up from the same batch of solution, the only difference being the addition of the potassium to the medium for M 22, after

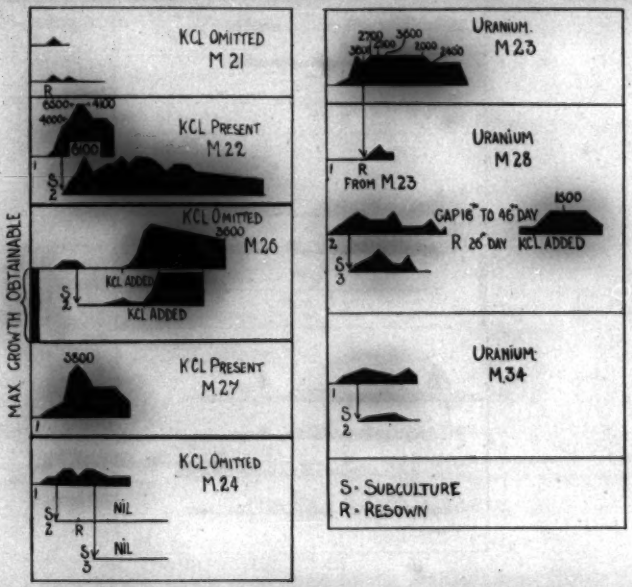

Fig. 9. Effect of potassium deficiency and of uranium substitution in quartz test-tube cultures.

having filled the M 21 tubes. M 22, 1 grew normally and gave a good subculture into M 22. On the other hand in M 21, 1, the colpidia merely survived a few days and then died. M 21, 2, was then sown, and the colpidia died. The latter was resown and the colpidia lived in a poor way for nine days without apparent division. Their movement in the potassium-free medium was very abnormal. They moved very slowly and were inclined to come to a complete standstill upon the bottom of the

tube. Possibly this may indicate a need of potassium for ciliary movement, but at any rate the difference is emphatic.

Another set of tubes—M 26, deficient in potassium, and M 27, the control—showed similar differences. M 27 grew well. M·26, 1, lived in a feeble way for six days after which a subculture was made into M 26·2,

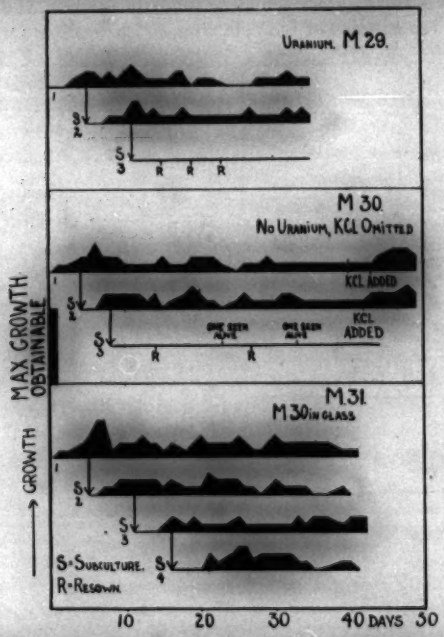

Fig. 10. Effect upon growth of (*a*) uranium addition to a potassium deficient culture in quartz with its control, (*b*) a similar potassium deficient culture in quartz without uranium, and (*c*) the same medium, *viz.* (*b*), in glass. NOTE. KCl was added to M 29·1 at end of period producing the rise of growth shown.

which survived in a feeble way for a few days. Upon the eleventh day after sowing M 26, 1, a trace of KCl was added to it. This was followed by a development of the colpidia up to 3600 per c.c. The experiments prove that potassium is essential.

Experiments were done in which the same potassium deficient medium was put up in both quartz and glass. The remainder of the

medium left after filling tubes M 21, was put up into glass tubes M 24. Fig. 9 shows the effect. There is growth in the first subculture.

M 30 and M 31 (Fig. 10) are another pair of experiments in which the effect of leaving out potassium in glass and silica respectively was tried. The difference in the character of the growth is quite striking, but M 30 in silica did better than the other non-potassium culture in silica (*viz.* M 21 and M 26). This can perhaps be attributed to the contact of the fluid with the glass measuring-flask, though the contact was more than one hour.

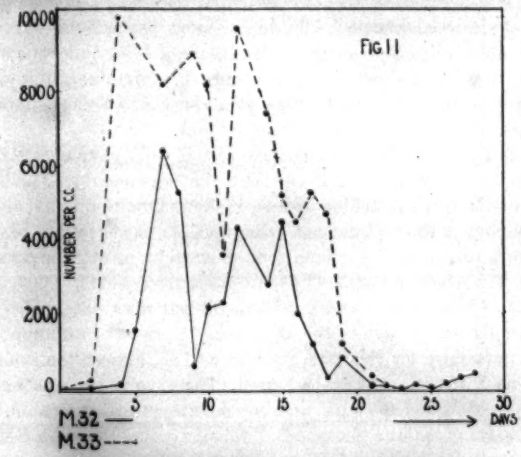

Fig. 11. Potassium deficiency. M 33. Jena glass flasks.
—— M 32 potassium deficient. - - - - Control containing potassium.

As a further check upon these results, 100 c.c. respectively of a medium containing potassium and a medium not containing potassium were put up in Jena flasks. The idea was that a difference, that would not show up in test-tubes, might show up in a flask where the ratio of glass surface to solution was smaller. The flasks were counted at regular intervals, and the results are shown in Fig. 11 (M 32 deficient in potassium, M 33). The ordinates represent numbers per c.c. and the abscissæ days. There is a marked difference between Exp. 5, and Exp. 6, though some potassium was apparently being obtained from the glass.

The evidence is that when we really reduce the concentration of

potassium below a certain point growth will not proceed. This brings the protozoon organism into line with the algæ molds and bacteria in this respect.

Uranium. As the result of his experiments upon the heart, Zwaardemaker(10) has concluded that the reason why potassium is essential to the normal activity of the heart is that it provides a source of radio-activity. In the absence of potassium, this source can be provided by other radio-active elements substituted in the right proportions. The idea that in the organism potassium provides a source of radio-activity is a fascinating one. Potassium is necessary for growth, and apparently in its absence colpidia do not move properly. If it could be shown that the function of potassium in the growth process could be taken on by other radio-active elements such as uranium, it would extend Zwaardemaker's view of the effect for the radio-activity of K to other functions of K than those given by him.

The cultures obtained in the course of this work gave a good opportunity of testing whether such a substitution was possible. The history of the work is worth sketching because it shows how an original observation in favour of the replacement being possible was shown to be wrong as the work progressed. A substitution of uranium nitrate for potassium chloride was originally made in four strengths: ·00002, ·0005, ·0001, ·0002 p.c. Good growth and good subcultures were obtained, in fact exceptionally fine growth in the ·0002 p.c. The ratio of uranium to the original potassium for this strength was as 1: 4, almost the same proportion as Zwaardemaker had used. The experiment was repeated with ·0001, ·0002 and ·0003 p.c. uranium nitrate, substituting ammonium phosphate for potassium phosphate. The same success was obtained, and good subcultures. Moreover the cultures were so striking that they could be easily separated from the others with the binocular microscope. A fresh experiment gave the result again. Upon measurement the colpidia were rather larger than normal.

A large number of tubes of two media were then prepared, one M 34*a* containing ·0002 p.c. uranium acetate, glucose ·04 p.c., ammonium lactate ·02 p.c. in place of ammonium glycerophosphate, and KCl; and the other M 34*b* ammonium glycerophosphate. The latter medium was the same as ammonium glycerophosphate medium with the exception that ·0002 p.c. uranium acetate was substituted for the potassium chloride. Subcultures from the uranium culture started these two new batches. M 34*a* grew exceptionally well. It has given consistently higher counts at its maximum than any of the other cultures.

On one occasion a culture containing 40,000 organisms per c.c. was obtained. The colpidia in it were also above the normal size, some, on one occasion were 17 scale div. Upon the medium 345 results have been consistently bad, not as good as the results with potassium M 1.

When the effect of potassium dissolved from the glass became known, it was clear that all the above media really contained potassium plus uranium. Some experiments were therefore done in silica tubes. The results are shown in Figs. 9, 10, in which uranium acetate ·0002 p.c. was substituted for KCl. The more carefully the experiments were done, the less evidence was there that uranium would replace potassium. M 23 grew in the first culture differing in this respect from its control M 21. In the second culture M 28, 1, growth was poor, suggesting the potassium deficient cultures. M 28, 2 (control M 27, 1) was sown from a uranium culture in glass containing therefore very little potassium.

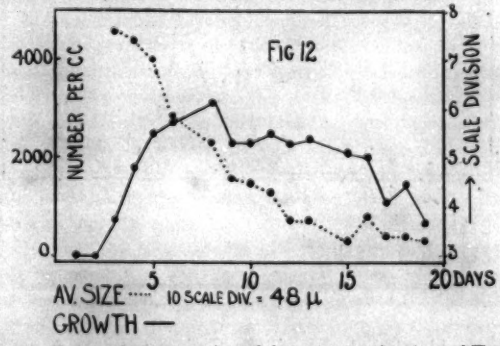

Fig. 12. Uranium containing culture deficient in potassium (control Fig. 3).

The reason why a potassium deficient culture was used in this case was that it might have been argued that it took some time to accustom the organisms to a change from potassium to uranium. (Zwaardemaker always perfuses with potassium-free Ringer first.) Growth was very poor as will be seen from the diagram. A subculture into M 28, 3, died. Before it died a subculture was made into M 29, 1. This dragged on a feeble life, until potassium was added to it, producing increased growth. When subcultured into M 29, 2, the organisms lived without growth so far as could be judged, and a subculture from them into M 29, 3, did not grow even when resown several times. Another experiment was done in

which quartz tubes were sown from a culture grown first in the uranium medium in glass test-tubes M 34, 1, 2, Fig. 9. In this case the organisms grew fairly well in the glass. When transferred to the quartz, there was a very slight growth in the first tube and none in the second.

Some experiments in Jena glass flasks upon a larger scale were also done by daily counting. M 35 is one of them. Fig. 12 shows the results. It will be seen that the behaviour of M 35 (Fig. 12) is very different to that of Fig. 3, which was a control to this experiment. The maximum growth obtained was never so great. Again M 23, and its control without potassium, Fig. 13, show not much difference between the potassium deficient and the uranium containing culture. At any rate there is no

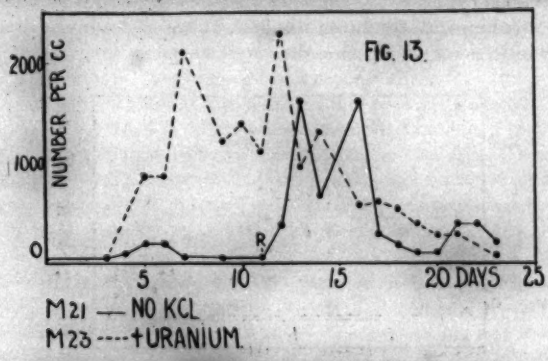

Fig. 13. Growth of culture deficient in potassium with and without the addition of uranium.

effect comparable to that of having potassium present. A further test was made in a silica flask to avoid any glass errors. A sample of M 29 was put up into a 100 c.c. silica flask. This was sown from the flask in M 35, Fig. 12, to allow for any possible acclimatisation effect that might be needed. The organisms as judged by counting lived for a few days without increasing in numbers.

The fact that subcultures cannot be made upon the uranium medium, where uranium is substituted for potassium, shows that uranium will not replace potassium in the growth process of this organism.

Is there any evidence for a partial replacement? A reference to the charts shows that M 23, 1, grew better than the control without potassium, M 21, 1. The same happened for M 28, 1, the control being M 26, 1.

There was not much difference, however, between M 29, 1, and M 30, 1. In the flask cultures the same thing tends to appear, a slightly better growth in the first subculture with the uranium than without it. The effect is not much but it is fairly constant, and unless there are traces of potassium in the uranium acetate sufficient to affect the cultures, it appears that uranium is having a slightly stimulating effect in the first subculture. This cannot be regarded as a replacement.

The fact that uranium cannot replace potassium in the growth process of *Colpidium colpoda* shows that Zwaardemaker's conclusions as to the function of K in connection with the heart and other specified functions cannot be extended directly to all biological functions of this element. If the radio-activity of K is the property which gives it peculiar value in the growth process, we should have to believe that this element is specially adapted for supplying the radiant energy. It would be necessary to postulate a two-fold function for potassium, (1) radio-activity, (2) chemical and physical. In the absence of any evidence upon this point, it seems better to conclude that K is of use *per se* in growth. This is a similar conclusion to that reached by J. Loeb(11) in a paper published after the completion of this work. Radio-activity will stimulate growth. Redfield(12) has shown that a moderate stimulus of β-rays stimulates division in Nereis eggs. A larger dose has the opposite effect.

The same type of observation has been made by Richards and Good(13) for Cumingia eggs.

The fact that the best cultures have been obtained by the addition of traces of uranium (M 34 *a*) suggest some stimulating effect. This has been observed by other workers empirically. Sasaki(14), for instance, added traces of uranyl phosphate (as a growth catalyst) to his media when working with *B. coli*. The increase in length of the protozoa in the uranium and potassium cultures suggests that uranium is stimulating growth, and slowing division rate.

Calcium. As in the case of potassium, experiments in glass test-tubes showed that it was not necessary to add calcium. The experiments were then repeated in quartz. The omission of calcium from the media set up in quartz tubes with silica distilled water was without effect. In one case organisms were taken through four successive subcultures in quartz and then into 70 c.c. of the calcium deficient medium in a quartz flask. After 16 days' growth in this flask there were 6000 colpidia per c.c. in the culture. The culture medium and protozoa were then evaporated in the quartz flask down to a bulk of $\frac{1}{2}$ c.c. and a spectroscopic wire test

for calcium done, which proved negative, indicating the presence of less than $\frac{1}{10}$ mgm. of calcium.

It therefore appears that down to the limits of experiment, it is not necessary to add calcium to get growth. As this is hard to believe, it seems well to consider the amount that might reasonably be expected to be necessary.

The length of a colpidium is about 60 μ and the breadth 20 μ (taking rather high values), from which it may be calculated that 100 c.c. of a 10,000 colpidia per c.c. culture cannot contain more than ·02 gm. of organism. The amount of potassium in blood corpuscles is given as about ·2 p.c., so that we might on the same basis expect to find ·2 p.c. × ·02 gm. of K in our culture, or ·00004 gm. K p.c. If the calcium percentage in the cell is of the same order as potassium, about ·00001 gm. p.c. Ca.

It is clear from this type of calculation that experiment upon the lines indicated can hardly settle whether calcium is necessary or not. It only seems remarkable that it should have been possible to demonstrate the need in the case of potassium. We must remember that there are two salt needs to consider: (1) the amount needed for structure, etc., inside the organism and (2) the amount needed in the environment immediately outside the organism for the maintenance of function at the surface layer.

Magnesium. Several experiments were made upon the effects of leaving out magnesium in glass test-tube cultures. One with M 36, Na_2SO_4 substituted for $MgSO_4$, shown in Fig. 14, is a good example. The cultures grew normally. Other experiments were M 37 in which $MgSO_4$ was left out, and which did not subculture well. A rise of concentration of $MgSO_4$ to ·01 p.c. was without harm. A repetition of the experiments under quartz conditions, gave distinctly poor results in magnesium deficient media, so that it appeared that the limit for magnesium deficiency had been reached.

Sodium. Fig. 7 shows the effect of leaving sodium out, ·02 p.c. ammonium chloride being substituted for NaCl, in media set up in Jena glass tubes. It will be seen that bad effects did not follow the cutting down of the sodium from ·06 p.c. NaCl to the traces not removed from the medium. In two media M 42, and M 43, the concentration of NaCl was ·01 p.c. and ·20 p.c. M 42 did well, showing a count of 9000 per c.c. in the second tube. M 43 did not subculture well and showed abnormal movement in the second culture (probably owing to change in the osmotic conditions).

In quartz a sodium-deficient culture was taken through two quartz

cultures and showed a growth of 6000 organisms per c.c. (full length) in the second tube. The limit for Na deficiency has therefore not been reached.

The inorganic requirements. Summarising the inorganic experiments, the experiments conducted in glass show that to obtain a growth of *Colpidium colpoda* ammonium, phosphate, chloride, must be added to a medium to get growth. The experiments in quartz, show that potassium

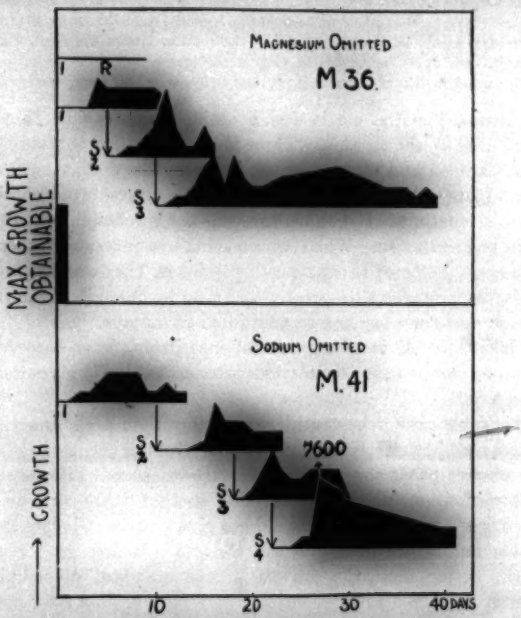

Fig. 14. Effect of magnesium and sodium deficiency in glass.

and magnesium must also be added. The necessity of sodium, of sulphate and of calcium has not yet been demonstrated. In all the experiments silica has been a constant factor.

We have therefore arrived at some knowledge of the basal inorganic constituents for this organism. Traces of other substances (in amounts below the accuracy of the work) are most probably required by the

organism, and when the work is conducted upon a larger scale, it may be found necessary to add other substances possibly supplied by the glass or water or air when working upon the small scale. All that has been proved is that from a practical standpoint, when working upon a scale not exceeding 500 c.c. cultures, if the above elements are present, any other impurities which may be required by the organism for its growth will be present too.

It is interesting to note that Lockemann (15) (for the tubercle bacillus) and Benecke and others (8) (9) (in the case of molds and other bacterial species) have found it unnecessary in some cases to add calcium and sulphur to their culture media. The results for colpidium agree with their experiments, but in view of the minute quantities of such substances possibly needed, further experiment is required to settle how far these deficiencies are real.

Nitrogen. The first media used contained amino acids. They seem to be well used by the organism. Three media were tried containing leucine as the nitrogen and carbon source, in strengths of ·05 p.c., ·1 p.c. and ·2 p.c. In the first two strengths good subcultures were obtained. In the last strength, the last subculture was not successful, probably owing to the concentration. When amino-acids are present in a culture, it would seem that growth starts rather quicker. They are also apparently used by the protozoa with greater ease than by bacteria of the *B. subtilis* group so that their use has an additional advantage.

The organic requirements. Several experiments have been done upon the carbon compounds necessary for growth. Table III gives a summary of the results.

This table cannot be considered as more than preliminary. Certain features may however be remarked upon. Growth has not been obtained upon sources of carbon under three carbon atoms. Life however was maintained upon all the media tried so that there was no obvious poisoning effect. Lactate alone did not give growth. Glucose alone gave variable but rather poor growth. Glucose + lactate gave remarkable growth, better than the growth in the standard glycerophosphate mixture.

For future studies upon organisms of this nature, it would appear most promising to work with glycerate, glycerophosphate, or glucose + lactate media, with perhaps the addition of a small amount of an amino-acid such as leucine. Before the ground has been more completely mapped out it would be unwise to attempt generalisations.

In spite of the different media on which this organism has been grown during the course of the 10 months' work upon it, the colpidium does not appear to have changed. Diet has altered the size temporarily;

TABLE III.

Carbon source	Growth	Medium Variations in place of ammonium glycerophosphate in standard medium
Nil (dissolved CO_2)	Nil	Am. phosphate ·06 p.c.
Monobasic acids.		
Formate (Na and Am.) ...	Nil	Am. phosphate ·0002 p.c., am. formate ·02, ·04, ·06 p.c., Na formate ·06 p.c.
Acetate (Na)	Nil	Am. chloride ·02 p.c., Sod. acetate ·03 p.c., sod. phosphate ·01 p.c.
Proprionate (Na)	Fair	Am. phosphate ·001 p.c., Na proprionate ·06 p.c.
Caproate (Am.)	Fair	Na ·001 p.c., am. caproate ·05 p.c.
Palmitate (Na)	Fair	NaCl ·02 p.c., AmCl ·04 p.c., Na palmitate ·06 p.c., am. phosphate ·001 p.c.
Monobasic hydroxy acids.		
Glycollate (Na)	Nil	NaCl ·02 p.c., am. phosphate ·001 p.c., am. chloride ·02 p.c., Na glycollate ·06 p.c.
Lactate (Am.)	Nil	Am. phosphate ·001 p.c., am. lactate ·05, ·1, ·2 p.c.
Glycerate (Am.)	Good	Na phosphate ·001 p.c., am. glycerate ·05 p.c.
Pyruvate (Na)	Good	Am. phosphate ·01 p.c., Na pyruvate ·05 p.c.
Dibasic acids		
Succinate (Na and Am.) ...	Nil	(1) Na phosphate ·001 p.c., am. succinate ·05, ·1, ·2 p.c. (2) NaCl ·02 p.c., am. phosphate ·001 p.c., am. chloride ·04 p.c., Na succinate ·06 p.c.
Oxalate (Am.)	Nil	Am. phosphate ·001 p.c., am. chloride ·02 p.c., am. oxalate ·06 p.c.
Tartrate (Am.)	Fair	Am. phosphate ·0002 p.c., am. bitartrate ·02, ·04, ·06 p.c.
Tribasic acids.		
Citrate...	Nil	NaCl ·02 p.c., am. phosphate ·001 p.c., am. chloride ·02 p.c., Na citrate ·06 p.c.
Various.		
Glycerophosphate (Am. or Fe)	Good	NaCl ·02 p.c., am. phosphate ·001 p.c., Fe glycerophosphate ·06 p.c.
Sugars, etc.		
Glucose	Poor	Na_2HPO_4 ·001 p.c., glucose ·06 p.c., am. nitrate ·01 p.c.
Glucose + succinate	Very poor	Na_2HPO_4 ·001 p.c., glucose ·04 p.c., am. succinate ·01 p.c.
Glucose + lactate	Very good	Na_2HPO_4 ·001 p.c., glucose ·04 p.c., am. lactate ·01 p.c.
Cane sugar	Good	Am. phosphate ·001 p.c., cane sugar ·06 p.c.

lack of food has led to a temporary diminution in size, but this has always been retrievable by fresh subculture into a standard medium, when organisms of average length have been obtained at the maximum of the new culture. Provided that the organism has grown, it has maintained the structure which it has reached in the course of its evolution. Change in chemical environment has not so far as can be ascertained produced real biological change. The factors, whatever they are, which

determine the structure of the organism, are apparently more deep-seated.

It is not possible to leave the subject without reference to the work upon the phenomenon of endomixis, worked out recently by Woodruff and Erdmann(16). According to their observations, paramœcia grown upon a uniform bacterial diet and isolated daily after division under conditions where conjugation is impossible, show periodically great changes in the nucleus. About every month a kind of splitting up of the nuclear material takes place followed by a reorganisation upon well determined lines. The process apparently replaces the sexual process, and after the phenomenon (termed endomixis), the paramœcia which divide, show much larger variation than the normal. In this way it is thought lies a means by which the organisms can adapt themselves to a fresh environment. Changing their environment causes endomixis to set in. It is of course not possible to say that this type of phenomenon has not occurred during the work done upon this colpidium. Many specimens have been stained and none of them have shown the characteristic changes described. It is rather likely that an alteration of osmotic environment would be the precursor to such a change and this factor has been kept fairly constant throughout.

Discussion.

In a current physiological text-book, the following statement is made: "the animal organism even in its lowest forms the protozoa, is satisfied with nothing less complex than glucose as a source of carbon (17)." It seems to be generally received opinion that most of the protozoa require bacteria as part of their food. This has been shown to be incorrect for *Colpidium colpoda*. A simple ammonium salt will supply it with nitrogen, and its carbon needs can be met with glyceric or proprionic acid. Up to the present the condition has not been realised in which this ciliate will utilise sources of carbon under three carbon atoms. Glycollic acid even has not been used, so that it is not merely a question of the presence of an oxidisable group. During the process of growth there is apparently a synthesis of fat and it is hoped to obtain some quantitative data upon this by working upon larger cultures.

It is clear that this protozoon organism is widely independent of its environment. From the biological standpoint this is largely what would be expected, as the colpidium must depend for its existence upon the chance and variable composition of rain puddles, etc. In these conditions from its nutritional needs it apparently is well equipped for

dealing with changing situations. What then is its place in nature? Apparently it is much more close to the autotrophic flagellate than many bacteria, but the absence of chlorophyll means that it is dependent ultimately upon the green cell for its needs. It is felt that the use of pyruvic and glyceric acids for growth, when contrasted with the non-utilisation of lactic acid suggests an unexpected relationship to the yeast cell, though the point cannot be pressed at present. The utilisation of ammonia by the cell in the synthesis of nitrogen compounds seems to be generally true for living cells (Boas(18)).

Comparative work upon the needs of other pure protozoal cultures should give material for fresh generalisations. Organisms of this nature are likely to prove of value for the study of several biochemical problems, such as the evolution of proteins, synthesis of fats, and possibly the relation between carbohydrate and fat. Though they do not multiply so quickly as bacteria, they have the great advantage of being easily kept under observation. Direct counts are easy and so are estimations of size. An extension of this type of work to the pathogenic protozoa should elicit more exact knowledge of the character of the toxins produced in disease, as well as giving a clear field for work upon life histories.

Summary.

1. Pure cultures in synthetic media of a ciliate protozoon, *Colpidium colpoda* (Ehrbrg) Stein, have been obtained from a single individual and studied for a year. Evidence is brought forward, which is regarded as conclusive that these cultures contain only the organism in question.

2. Upon a medium consisting of glass distilled water, calcium, potassium and sodium chlorides, magnesium sulphate, and ammonium glycerophosphate, test-tube cultures have been kept going by frequent subculture, without diminution in the size of the organisms. Rather better results are got by substituting glucose and ammonium lactate, as nitrogen and carbon sources.

3. By counts and measurements of size, it has been found that the average maximum for growth under these conditions is 8000–10,000 organisms per c.c. The growth curve shows three phases: (1) a rise to a maximum number, (2) a more gradual fall to (3) a plateau, during which life may continue for some time. Phases (1) and (2) last about 12 days. During phases (2) and (3) the organisms diminish in size. During phase (2) temporary rises in the number of organisms may take place.

4. Experiments upon the omission separately of the medium constituents, showed that ammonium, phosphate and chloride could not be

omitted from glass test-tube cultures without stopping growth. Phosphate deficiency in the medium led to apparent disintegration. The omission of potassium and magnesium in glass cultures was without effect, but in quartz test-tube cultures potassium deficiency led to failure of movement and death of the cultures. Magnesium deficiency also inhibited growth. No effect was produced by omitting the sodium, calcium or sulphate separately from the medium, though it is not claimed that traces of Na, Ca or S were not still present.

5. Uranium salts cannot be substituted for potassium salts in the growth process, thereby proving that the biological value of potassium is not solely a question of radio-activity.

6. Amino-acids will serve as nitrogen source in place of ammonium salts.

7. Growth has not been demonstrated on carbon sources containing less than three carbon atoms in the molecule. Glycerate is used, but not lactate or citrate.

I am indebted to Professor Hopkins for his kind criticism and to many others in the laboratory for the loan of chemicals, I am also much indebted to my assistant, H. Mowll, for his patient and skilful help throughout the experiments, and for drawing the diagrams.

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THE REPLACEMENT OF POTASSIUM BY URANIUM IN PERFUSION OF THE HEART. By H. ZWAARDEMAKER.

(*From the Physiological Laboratory, Utrecht.*)

In previous papers(1) I have shown that uranium or any other radio-active element can be substituted for potassium in Ringer's fluid in perfusion without disturbing the automaticity of the heart of the frog and the eel, provided the amount of the element substituted is radio-equivalent to that of the potassium in the fluid. I pointed out the amount of uranium required for effective substitution was not the same in summer and in winter frogs, *i.e.* that there are factors which increase or decrease the effect of radio-activity. Recently Clark(2) has been unable to confirm my results. The absence of effect of uranium when substituted for potassium, which he found, is I think due to his having overlooked the considerations I have just mentioned.

In winter the dose of potassium in a perfusion fluid that does not return to the heart may vary without loss of automaticity from about ·003 to ·06 p.c. KCl. In summer the range is much smaller, *viz.* about ·001 to ·003 p.c.; on a warm day it may be even less, as ·0005 to ·001 p.c. Consequently the dose of uranium must vary within corresponding limits. The amount of calcium has also great influence; in Clark's experiments, the $CaCl_2$ content was low, *viz.* ·014 p.c. I have made some further experiments using Ringer's fluid of the composition used by Clark in order to determine the cause of the difference in result.

For the frog's heart I employed Kronecker's cannula, the record then being that of the ventricle only, which has the advantage of allowing small hearts to be used. In Fig. 1 the perfusion was at first with an inactive fluid, *i.e.* with a Ringer's fluid containing no radio-active atom. At the signal mark Ringer's fluid containing ·0025 p.c. KCl was substituted for this. The beats were at first small and it was concluded that the proper amount of K had not been given. The KCl content was then reduced to ·0015 p.c. The beginning of Fig. 2 shows the beats thus obtained; at the signal mark the fluid was replaced by another which contained ·0005 p.c. uranyl nitrate instead of potassium. It will be seen that automaticity proceeded undisturbed. Without a hitch the heart passed from a potassium-beat to a uranium-beat. Although the experi-

ment was made in November, the laboratory animal was still in a summer condition; this was also indicated by the lively behaviour of the companion frogs. We find here low potassium dose, low uranium dose, and absence of intermediate standstill at the moment of transition.

The absence of intermediate standstill is however not constant. It may happen that at the moment of transition from the one fluid to the other, a temporary standstill, which continues for some minutes, is inserted. This is instanced in Fig. 3 at the transition from the potassium-beat (beginning of the figure) to the uranium-beat (end of the figure). In the middle a standstill of 6 minutes, commencing almost directly after the transition, its time being marked by the signal. This phenome-

Fig. 1.

Fig. 2.

Fig. 1. Passage from an inactive fluid to a fluid containing 25 mg. of KCl. Time intervals, 3 min.

Fig. 2. Substitution of 15 mg. of KCl per litre by 0·5 mg. of uranyl nitrate.

non, which occurs constantly in winter when passing from a perfusion with a light radio-active metal to that with a heavy one, or the reverse, I have termed the radio-physiological paradox(3). It appears also when in winter we pass from a light radio-active metal to a perfusion with potassium-free Ringer's solution, to which some emanation has been added. Radium-emanation, however, is a chemically indifferent atom. The facts show the correctness of my conclusion that the paradox is in its origin radio-active.

The fact that in summer the radio-physiological paradox is sometimes met with, and is not at other times, has long engaged my attention. It has lately appeared that the presence of sensibilising substances has

something to do with it. Their presence compels us to make the dose of the radio-active atoms exceedingly small, which renders the fall of the concentration, at the moment of transition from light to heavy metal, very slight. I ascribe the absence of paradox with low summer-doses to the rather slow diffusion of the new atoms in the lacunæ of the heart perfused with the old atoms. This hypothesis is substantiated by what is shown in Fig. 4, in which a heart is represented in its automaticity, which had already been pulsating for hours at a stretch, now with potassium, now with uranium. It had already become slightly hypodynamic and the balancing(4) between the K-, resp. uranyl-ions on one

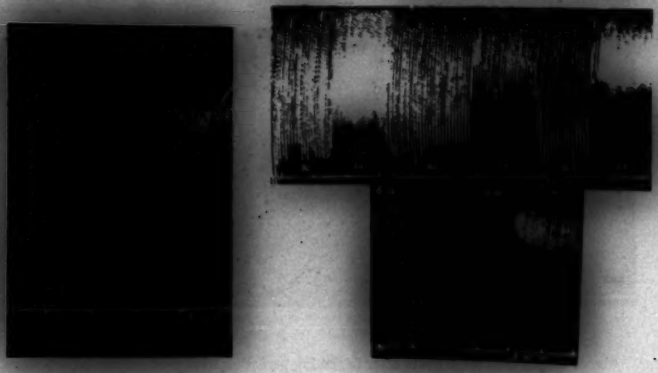

Fig. 3. Fig. 4

Fig. 3. Standstill during the transition from the fluid containing 25 mg. of KCl to the one containing 0·45 mg. uranyl nitrate. Time intervals, 3 min.

Fig. 4. See text. Time intervals, 3 min.

side and the Ca-ions on the other, could by no means be considered complete. In consequence of this the tone of the heart increases more during the uranium-beat than during the potassium-beat. As the reader will observe, the paradox is entirely lacking at a given period (upper half of the figure). An hour later, after alternate perfusion with potassium and uranium, the complete paradox appeared again. I take it that part of the sensibilising substances have been washed out, that in consequence of this the tendency of the heart to resume its automaticity on radio-active impulses has diminished, and that finally the phenomenon of standstill at the transition, so common in winter, has revealed itself.

In summer, pulsation on small uranium doses of hearts with adequate vitality will continue for many hours. Fig. 5 shows a heart that had been pulsating in the morning (upper part of the figure) for a couple of hours on ·0005 p.c. uranyl nitrate, and in consequence of this prolonged beating, showed a slight slackening of rhythm. The lower part of Fig. 5 shows the beat 3 hours later; during this time the heart had dragged the lever, which magnified 20 times, over the smoked paper. This rendered the heart hypodynamic, but the automaticity, as will be seen, was not impaired and the frequency was scarcely decreased.

The experiments on the eel's heart were made with a Symes cannula, the whole heart being taken. In this, the summer dose of

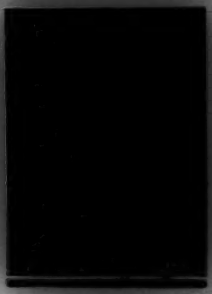

Fig. 5.

Fig. 6.

Fig. 5. Heart beat some hours after beginning perfusion with fluid containing 0·5 mg. of uranyl nitrate. Time intervals, 3 min.

Fig. 6. Eel's heart. Withdrawal and addition of uranium to the perfusion fluid.

uranium could be higher, thus in Fig. 6 the fluid contained ·002 p.c. uranium nitrate. Fig. 6 also shows that by an inactive fluid, *i.e.* a Ringer's solution containing no radio-active atom, the cardiac action is arrested. The signals in the lowermost line mark the moments when the uranium fluid (beginning of the figure) is substituted by an inactive fluid (middle of the figure) and when this is replaced again by uranium fluid (end of the figure). The standstill is completed $1\frac{1}{2}$ minutes after the uranium perfusion has stopped; the automaticity is restored 1 minute after the uranium perfusion has recommenced. Needless to say that for the rest the state of the heart was subject to the same conditions; the temperature remained constant; so did the hydrostatic pressure (Mariotte's flasks), the osmotic pressure, the air bubbling, the H-ion

content. Only the balance of the $\frac{UO_2}{Ca}$-ions was disturbed, first a little, afterwards completely. It is to this disturbed balance, therefore, and not to the absolute absence of a radio-active atom, that some might attribute the standstill. This is quite reasonable, provided it be borne in mind that in the given case there is no question about a balance between univalent and bivalent ions, as with Sidney Ringer and J. Loeb, but about a balance between a radio-active element and the element calcium (*casu quo* replaceable by strontium or barium, but not by magnesium) (5). In formula, a balance not between $\frac{Na+K}{Ca+Mg}$ but between $\frac{K+(UO)_2+Th}{Ca+Sr+Ba}$. In collaboration with T. P. Feenstra I have placed the latter at the side of the radio-physiological antagonism without any identification of the two. The explanation, though reasonable, is not in accord with other facts. The experiments with emanation and with radiation show that radio-activity and not a disturbance of ionic balance is the strict condition for automaticity.

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STUDIES ON MUSCULAR CONTRACTION. III. The influence of extension and temperature on the heat production in the muscle twitch.

By Y. DOI.

(*From the Physiological Laboratory, Cambridge.*)

In previous papers(1) I found that the two expressions of the energy output in muscular activity, *i.e.* the tension developed in an isometric contraction, and the maximal work done in an actual shortening, were influenced in a similar way by extension and temperature. The heat production of the muscle on isometric contraction is another function of the energy output in muscular activity, and it is interesting, therefore, to see if the extension and temperature affect it in the same way as they affect the tension and the maximal work.

According to A. V. Hill and Hartree(2), the process of the tetanic muscular contraction is divided into four phases, *i.e.* the development of the mechanical response, its maintenance, its disappearance, and finally the oxidative recovery process, each of these phases being accompanied by a certain heat production. On isometric contraction the potential energy which develops in the first phase is the source of the heat production in the third phase, and it is likely that in the latter phase all of the potential energy is degraded into heat and is not restored to the muscle. If so, the sum of the heat productions of the first three phases corresponds to the total initial energy output in muscular activity. It is obvious that in the single twitch, which I propose to treat here, the second phase does not exist and the first phase is directly followed by the third phase.

It was found by Weizsäcker(3) that, when a muscle was kept fixed at a certain extension, the heat produced in its activity was greater at a lower temperature than at a higher. This conclusion was the reverse of that of Fick(4), but Fick's conclusion was not supported by his own experiments. On the other hand, Evans and Hill(5) found that the heat production was maximal at a certain extension. It is worth while, therefore, to study, whether the heat production at different extensions and temperatures has a similar behaviour to that of the tension developed or of the work done.

Methods. The methods of measuring the heat production of a

muscle in an isometric contraction was recently improved by Hill and Hartree(2). I have followed their method and by the kindness of Mr Hartree I have been able to use the apparatus used by them; it will be superfluous, therefore, to describe the details, and only the following points need be mentioned.

The thermopile[1] used has 60 pairs of iron-constantan junctions, and is 16 mm. long, the distance between the two stimulating electrodes being 20 mm., so that measurement of the extension of a muscle as short as 20 mm. is possible. The stimulus applied to the muscle was always a single maximal break induction shock.

The heat produced by the muscle was estimated by the maximum deflection of the galvanometer, so that it is the sum of the heat productions of the first and third phases. As the contraction is a single one, and not tetanic, it is obvious as stated above, that the heat production on the second phase does not exist. The amount of heat is measured in absolute units (calories). The heat equivalent of the galvanometer deflection is calculated as follows. I have assumed that the velocity of heat conduction along the length of the muscle is negligible compared with that of heat conduction into the thermopile from the muscle in contact with the thermopile. Suppose c calories of electric energy (measured in heat units) is given to the killed muscle substance between the two electrodes (which are 20 mm. apart) and that d mm. of galvanometer deflection is observed. The heat produced in the muscle substance which is actually in contact with the thermopile (16 mm. long) is $c \times \frac{16}{20}$, so that the heat equivalent of the galvanometer deflection is given by

$$1 \text{ mm.} = c \times \tfrac{16}{20} \times \frac{1}{d} \text{ calorie.}$$

Now when deflection D is observed in the case of an actual twitch, the heat produced in the whole muscle is

$$\text{Heat equivalent} \times D \times \frac{l}{16} = \frac{cDl}{20d} \text{ calorie,}$$

where l is the length of the muscle in the case considered.

The procedure of the experiments is as follows. A double sartorius preparation of a frog is mounted on the thermopile. The piece of bone, to which the pelvic ends of the muscles are attached, is clamped at the bottom of the chamber of the thermopile. The other ends of the muscles, which are directed upwards, are attached to a straight wire, which leads upwards through the tube of the chamber and is fixed to a tension lever.

[1] This thermopile is made by my friend Dr R. Shōji according to the design of Mr Hartree.

The tube of the chamber is clamped firmly and can be lowered by means of a screw for any desired distance accurately to $\frac{1}{10}$ mm., so that the muscle can be held rigidly at the desired extension. Water at 5° C. is put in a vacuum flask, in which the chamber of the thermopile is immersed. The chamber is filled with oxygenated Ringer's solution of 5° C. which is replaced by oxygen gas after 10 minutes. When the galvanometer has settled down, *i.e.* when the thermopile gives no thermal current (a state obtained 30–50 minutes after the chamber is filled with oxygen), the muscle is stimulated and the heat production is measured. This measurement is repeated with a slightly stronger stimulus to ensure that the contraction is really a maximal one, and that the heat production is the same in both measurements. Then the water in the vacuum flask surrounding the chamber is replaced with the water of 15° C., the chamber is filled with the oxygenated Ringer's solution at 15° C., which is replaced with oxygen after 10 minutes, and when the galvanometer has settled down, the heat production on excitation is estimated as before. Next the temperature of the arrangement is brought again to 5° C. and the heat production is estimated once more. In most cases the heat production observed at this third estimation, at 5° C., is slightly smaller than that at the first estimation which was also at 5° C., and the mean of the two estimations is taken. The difference between the two estimations at 5° C., however, is much smaller than that between the first estimation (at 5° C.) and the second (15° C.). When the former difference is rather larger and comparable to the latter, such an experiment is rejected, as it shows that fatigue or some injury is affecting the muscular activity. Now such measurements at two temperatures are repeated at the next extension, which is longer by a few mm. than before and so on. After a series of the measurement at various extensions has been done, the muscle is killed with chloroform vapour and the heat equivalent of the galvanometer deflection is estimated. This heat equivalent must theoretically be the same at different temperatures, the extension being the same, and I have confirmed this in preliminary experiments; but it is not the same at different extensions of a single muscle, as the thickness of the muscle on the thermopile is different at different extensions, so that it must be estimated at each extension, at which the heat production is measured. Fortunately I found that this heat equivalent of the galvanometer deflection is a linear function of the extension and its gradient is the same for different muscles, so that in some experiments I estimated it at one or two extensions and calculated the values at other extensions.

Results and conclusion. Eight experiments were made all of which gave similar results, so that one example given in Table I and Fig. 1 is sufficient to represent them all. In this example the heat equivalents of the galvanometer deflection were actually estimated at each extension.

TABLE I.

Relative length of extended muscle	Heat calorie	
1·19	$4·10 \times 10^{-7}$	$3·03 \times 10^{-7}$
1·27	4·91	3·36
1·35	5·48	3·59
1·42	5·64	4·02
1·50	5·50	4·71
1·58	4·99	4·04
1·66	4·04	3·94

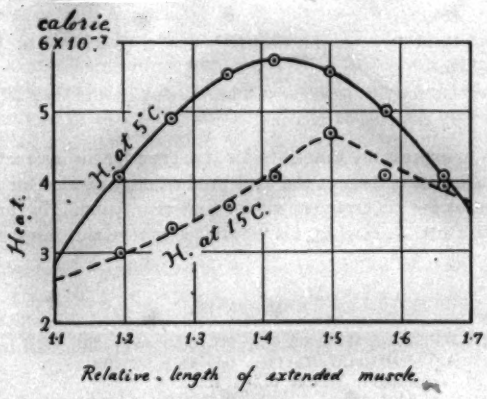

Fig. 1.

The general type of the heat-extension curve is similar to those of the tension-extension curve and of the maximal work-extension curve, and the influence of temperature on those are also alike. The heat production is maximal at a moderate extension. Before this optimal extension is attained, the heat production increases, and beyond this optimal extension, it decreases with increasing extension. This optimal extension of a single muscle is a few millimetres shorter at 5° C. than at 15° C. The heat production at 5° C. is larger than that at 15° C. at every extension, but beyond this optimal point the decrease of the heat production with increasing extension is much steeper at 5° C. than at 15° C.,

and it was found that at a certain extreme extension the heat productions at both temperatures coincide with each other, so that it is probable that at a further extension, the heat production at the lower temperature may become less than that at the higher, though such an extreme case was not studied in my experiments. As to the relation between heat production and tension developed, the results obtained are not sufficient to deduce a reliable conclusion and further experiments are required.

Summary.

The statement is confirmed that the heat production reaches a maximum at a certain moderate extension. Before this optimal length the heat production increases, and beyond this it decreases with increasing extension.

The optimal extension of a muscle is shorter at a lower temperature than at a higher.

The heat production is larger at a lower temperature than at a higher except at very extreme extensions.

I wish to express my thanks to Mr Hartree for his instruction and advice on the estimation of the heat production, and also for his kind permission to use his arrangement for heat estimation. My thanks are due also to Dr A. V. Hill for his valuable advice in this research.

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STUDIES ON RESPIRATION AND CIRCULATION IN THE CAT. I. The influence of an acute anoxic anoxæmia on respiration and circulation.

By Y. DOI.

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The present research was undertaken primarily for the purpose of finding out whether a reduction in the partial pressure of oxygen in the inspired air necessarily leads to a change in the volume of blood which traverses the body in unit time ("minute-volume"). That an increase of the minute-volume should result from a reduction in inspired oxygen is not unreasonable for it would tend to redress a deficiency in the quantity of oxygen present in each cubic centimetre of blood by the contribution to the body of a greater number of cubic centimetres of the partially saturated blood in unit time.

Method. The experiments were carried out on anæsthetised cats and the results can only be regarded as applicable to animals in this condition. Unless otherwise stated the cat was anæsthetised with urethane; volatile anæsthetics were avoided owing to the possibility of their producing disturbances of the gaseous pressure in the blood gas analysis apparatus.

A tracheal cannula with two branches was inserted, one limb being connected through an inspiratory valve to a Douglas bag containing the atmosphere to be breathed (if this was not atmospheric air). The other limb of the tracheal cannula was connected with a T-tube, which in turn communicated (*a*) with a Marey's tambour in order to record the respiration; (*b*) with an expiratory valve which led to a three-way tap. Through this tap the expired air must therefore all pass, but according to the disposition of the stopper the expired air may be passed out into the external air or may be collected in a rubber bag. Analysis showed that so far as the oxygen was concerned the air might be kept in such a bag for a sufficient time to allow of subsequent analysis. The carbonic acid analyses are probably less accurate, but not so inaccurate as to be valueless.

The inspired and expired air were analysed in Haldane's gas analysis apparatus. The volume of air collected was measured. From these data the quantity of oxygen taken up (and CO_2 expired) per minute was

calculated, and also the total ventilation per minute. By counting the respiration a number of collateral data, such as the ventilation per respiration, may also be obtained.

Whilst the expired air was being collected a sample of the general venous blood was withdrawn from the right ventricle by the method of direct puncture with the needle of a 1 c.c. hypodermic syringe. For this purpose the cat should be on its right side. A small incision was made in the skin at the position of the puncture. In preliminary experiments, this procedure was carried out on the table of an X-ray apparatus, the position of the heart being of course then easily seen. In most cases it was very easy to judge by the colour of the blood whether it was a venous sample or no. The point, if in doubt, was cleared up subsequently by analysis. Immediately after the collection of the venous blood a sample of arterial blood was withdrawn from the femoral artery by means of the same syringe as was used for the collection of the venous blood. The needle used for piercing the arterial wall was very fine, so that a slight compression with the finger or with cotton wool was sufficient to stop the bleeding after the withdrawal of the needle. The syringe used was long and of narrow bore and the end of its glass plunger was sharply cut so that it was possible to measure 1 c.c. with an error of at most ·002 c.c.

The amount of oxygen absorbed in each 1 c.c. of the arterial and venous blood respectively was measured by means of Barcroft's differential blood gas apparatus, and then the hæmoglobin content of each blood sample was measured colorimetrically by means of Dubosq's colorimeter, converting Hb to CO-Hb. A sample of arterial blood, taken at the beginning of the experiment, was used as the standard and its Hb concentration was arbitrarily taken as 100. Oxygen capacity of this standard sample is determined by ferricyanide method in a differential apparatus. The data of oxygen absorbed in the blood samples are corrected in regard to the variation of Hb concentration, so that these data are compared for the same amount of Hb, and not for the same volume of the blood, though this correction term is not very large. The oxygen content and the percentage saturation of the arterial and venous blood are calculated from these data.

The "minute-volume" F is calculated by the formula $F=\frac{O}{A-V}$, where O is the corrected volume of oxygen taken up by the animal per minute, and A and V are the oxygen content of the arterial and the venous blood respectively. From minute-volume and frequency of the heart beat the output of the heart for each beat is calculated.

To pass to the actual course of an experiment: the animal was first allowed to breath atmospheric air and whilst doing so a determination of the minute-volume was made. Then air of lower oxygen pressure was administered and left for 10–15 minutes in which time the animal settled to a more or less steady condition.

These measurements were repeated several times with various atmospheres and finally a determination was again made with the animal breathing atmospheric air, in order to see how nearly it returned to its original condition. The time which must elapse before the cat gets into apparent equilibrium with a low oxygen-pressure differs for different pressures, the lower the oxygen-pressure, the sooner the animal responds to it. If the oxygen-pressure be very low, after a certain period of tolerance in which the cat appears to have accommodated itself to the new conditions it seems to become suddenly weaker and when cats are subjected to a pressure of 14 p.c. for over thirty minutes, recovery, on subsequent administrations of atmospheric air, becomes very slow and difficult, and on occasions has proved impossible. The lower the oxygen-pressure in the inspired air the sooner is it necessary to make the experimental determinations. It is very important, too, that before another air of lower oxygen-pressure is applied, the animal should be allowed to breathe the normal air for 30–60 minutes, in order to get rid of the effect of the former low oxygen-pressure.

The blood-pressure and body temperature were recorded, and every possible care was taken to keep them unchanged. The amount of blood taken for samples was substituted in each determination with the same amount of 6 p.c. gum-saline solution. At the moment of heart puncture a slight fall of the blood-pressure is sometimes seen, but in the most cases it recovers spontaneously, and when it does not, gum-saline solution was injected to keep it constant.

The results obtained from five experiments made on urethanised cats agree with each other quite satisfactorily; full details need only be given for one, as is done in Table I.

The results are summarised as follows:

1. *Oxygen taken in by the animal.* The amount of oxygen taken in per minute is practically constant for different oxygen-pressure of breathing air. In no case is any evidence of increase observed. In the example in Table I 20·15 c.c. of oxygen were taken in per minute, when the normal air was breathed, and 19·95 c.c., 20·20 c.c. and 19·67 c.c. with air containing 18·70 p.c., 16·55 p.c., 13·31 p.c. of oxygen respectively. Finally the normal air was again introduced, and the amount of oxygen

TABLE I. Urethanised cat. 3·1 kilo.

P.c. of O_2 in air breathed in	20·96	18·70	16·55	13·31	20·96
P.c. of CO_2 in air breathed in	·04	·03	·02	·01	·04
Blood-pressure (mm. Hg)...	153	151	151	148	150
Body temperature (C.)	37·1	36·9	36·9	36·7	37·0
Pulse-rate per minute	160	180	190	202	172
Frequency of respiration	28	30	33	33	29
P.c. of O_2 in air breathed out	16·63	14·76	12·83	9·66	16·78
P.c. of CO_2 in air breathed out	3·41	3·14	3·00	2·97	3·34
Volume of air breathed out per minute (c.c.)	430	480	515	520	440
O_2 taken up in c.c. per minute (0° C. 760 mm., dry)	20·15	19·95	20·20	19·67	19·90
CO_2 given out in c.c. per minute... ...	14·80	14·91	14·35	15·81	14·85
True respiratory quotient...	·778	·792	·802	·806	·788
Difference between O_2 in 100 c.c. arterial and venous blood	5·48	5·56	5·58	5·76	5·55
Minute volume	367	359	362	342	358

Oxygen capacity (c.c.), 15·60.

P.c. of O_2 in inspired air	Hb. concentration		O_2 absorbed by 100 c.c blood		O_2 content in 100 c.c.		P.c. saturation	
	art.	ven.	art.	ven.	art.	ven.	art.	ven.
20·96	100·0	102·4	1·65	7·13	13·95	8·47	89·4	54·3
18·70	102·1	99·8	2·08	7·64	13·52	7·96	86·7	51·0
16·55	100·9	99·9	2·43	8·01	13·17	7·59	83·3	47·1
13·31	96·1	94·6	2·84	8·60	12·76	7·00	81·8	44·8
20·96	97·5	97·9	1·86	7·41	13·74	8·19	88·2	52·6

taken in increased and became 19·90 c.c., which shows practically the same as the first. It is clear that the progressive increasing action of the anæsthetic did not affect the results. The other results are summarised in Table II.

TABLE II.

Oxygen taken in by the animal per minute (c.c.).

P.c. of O_2 in air breathed	Exp. I	Exp. II	Exp. III	Exp. IV	Exp. V
20·96*	14·37	23·30	22·70	11·40	20·03
18·70	—	—	—	—	19·95
18·62	—	—	—	11·10	—
18·15	14·50	—	—	—	—
17·98	—	—	21·50	—	—
17·90	—	23·10	—	—	—
16·55	—	—	—	—	20·20
15·65	—	—	—	10·84	—
15·40	—	—	21·90	—	—
13·87	—	—	23·70	—	—
13·70	—	—	—	11·41	—
13·60	—	22·60	—	—	—
13·31	—	—	—	—	19·67

* Average of the first and the last determination is given.

2. *CO_2 given out by the animal.* The amount of CO_2 given out slightly increases with low oxygen-pressure.

3. *Volume of one respiration.* The total respiration per minute increases with low oxygen-pressure, as has been found by many other

workers. In the example in Table I it was 430 c.c. per minute, when the inspired air was normal, with air of low oxygen-pressure of 18·70 p.c., 16·55 p.c., and 13·31 p.c., it was 480 c.c., 515 c.c. and 520 c.c. respectively. The number of respirations, too, increases in most cases with low oxygen-pressure, but in a few cases this increase is very slight. The volume of one respiration therefore is irregular, but in most cases it increases slightly with low oxygen-pressure. The results of five experiments are summarised in Table III.

TABLE III.

Exp.	I		II		III		IV		V	
P.c. of O_2 in air breathed in	Vol. of respiration p.m. c.c.	Vol. of one resp. c.c.	Vol. of resp. p.m. c.c.	Vol. of one resp. c.c.	Vol. of resp. p.m. c.c.	Vol. of one resp. c.c.	Vol. of resp. p.m. c.c.	Vol. of one resp. c.c.	Vol. of resp. p.m. c.c.	Vol. of one resp. c.c.
20·96*	310	7·84	445	13·73	353	11·78	209	5·88	435	15·27
18·70	—	—	—	—	—	—	—	—	480	16·00
18·62	—	—	—	—	—	—	223	5·72	—	—
18·15	365	8·92	—	—	—	—	—	—	—	—
17·98	—	—	—	—	360	10·90	—	—	—	—
17·90	—	—	590	15·95	—	—	—	—	—	—
16·55	—	—	—	—	—	—	—	—	515	15·65
15·65	—	—	—	—	—	—	240	5·85	—	—
15·40	—	—	—	—	430	12·65	—	—	—	—
13·87	—	—	—	—	465	12·90	—	—	—	—
13·70	—	—	—	—	—	—	243	5·94	—	—
13·60	—	—	630	16·16	—	—	—	—	—	—
13·31	—	—	—	—	—	—	—	—	520	15·75

* Average of two determinations is given.

4. *Percentage saturation of oxy-hæmoglobin in the arterial and venous blood.* This decreases with decreasing oxygen-pressure in the breathed air. It is found, however, that the decrease in arterial and venous blood is nearly parallel, so that the difference of oxygen-content in the arterial and venous blood is fairly constant. In the example given the difference is 5·48, 5·56, 5·58 and 5·76 c.c. in each 100 c.c. blood, when the percentages of oxygen in the breathed air are 20·96, 18·70, 16·55 and 13·31 respectively.

5. *Minute-volume.* The volume of blood passing through the lungs per minute undergoes practically no change when an air of lower oxygen-pressure is breathed. This result is rather remarkable, as it disproves, so far as cats under urethane are concerned, the conception that the effect of the lower oxygen-pressure in the air breathed is compensated by the increase in the rate of blood flow through the lung. The figures given in Table IV, Exp. v show 363, 359, 362 and 342 c.c. per minute, corresponding respectively to the 20·96, 18·70, 16·55 and 13·31 p.c. oxygen in the inspired air. The variation between these figures is within the limit of

experimental error, or might be due to slight change in the blood-pressure which is caused by some inevitable reflex from sensory nerves of the skin.

Table IV. Minute-volume in c.c.

P.c. of O_2 in air breathed in	Exp. I	Exp. II	Exp. III	Exp. IV	Exp. V
20·96*	303	378	431	225	363
18·70	—	—	—	—	359
18·62	—	—	—	217	—
18·15	296	—	—	—	—
17·98	—	—	411	—	—
17·90	—	361	—	—	—
16·55	—	—	—	—	362
15·65	—	—	—	206	—
15·40	—	—	407	—	—
13·87	—	—	416	—	—
13·70	—	—	—	211	—
13·60	—	347	—	—	—
13·31	—	—	—	—	342

* Average of two determinations is given.

6. *Pulse rate and output of the heart.* In agreement with many other authors' results the pulse rate increases with decreasing oxygen-pressure in the inspired air. And as a necessary result of this accelerated pulse and the constancy of minute-volume, it obviously follows that the output of the heart decreases, when an air of low oxygen-pressure is introduced.

Table V. Output of each heart beat in c.c.

20·96*	2·00	2·33	2·43	1·31	2·19
18·70	—	—	—	—	2·00
18·62	—	—	—	1·20	—
18·15	1·78	—	—	—	—
17·98	—	—	2·28	—	—
17·90	—	2·01	—	—	—
16·55	—	—	—	—	1·90
15·65	—	—	—	1·08	—
15·40	—	—	2·16	—	—
13·87	—	—	2·13	—	—
13·70	—	—	—	1·03	—
13·60	—	1·87	—	—	—
13·31	—	—	—	—	1·70

* Average of two determinations is given.

Summary.

The statement is confirmed that, when the cat is subjected to a lower oxygen-pressure than the ordinary atmospheric air, the volume of blood passing through both lungs per minute is the same as that when the breathed air is normal, regarding the hæmoglobin concentration as the same.

After a short exposure to a low oxygen-pressure, the cat takes up the same amount of oxygen as that in the normal air.

On an acute anoxic anoxæmia the volume of the respiration per minute increases.

When the cat is exposed to a deficiency of oxygen the frequency of respiration and pulse increases, and the output of each heart beat decreases.

The decrease of the percentage saturation of oxyhæmoglobin in the arterial and venous blood is nearly parallel in the state considered, so that the difference of oxygen content of the arterial and venous blood is fairly constant.

In conclusion I wish to express my warmest thanks to Mr Barcroft for his suggestion of this problem and for his kind and valuable advice through the course of these experiments. I am indebted also to Prof. Langley, in whose laboratory these experiments were made. Much of the apparatus used was bought with a grant from the Government Grant Committee of the Royal Society.

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ON THE AMOUNT OF HEAT LIBERATED BY BACILLUS COLI WHEN GROWN IN THE PRESENCE OF FREE AMINO-ACIDS.

By C. SHEARER, F.R.S.

In the course of some experiments on the estimation of the amount of heat liberated by B. coli when grown on various culture media, it was noticed that it produced little or almost no heat when grown on tryptic broth. This broth is prepared from casein by digestion with pancreatic extract and contains variable amounts (usually 2 p.c.) of a number of amino-acids. It was found that if the casein was well digested, the resulting fluid grew B. coli extremely well, and the heat liberated for a definite quantity of growth was always 6–8 times less than that produced from the same growth on ordinary nutrient glucose peptone broth.

It would seem that if the bacteria were supplied with sufficient free amino-acids ready to hand, surprisingly little or almost a negligible quantity of energy was lost in the process of their being built into the living substance. A comparison of broths digested for 1 week and those digested for a longer period (4–8 weeks) showed that the one week's digest always gave a much greater amount of heat for the same growth than that digested for 4 weeks. So within limits it seemed that the amount of heat liberated by B. coli when grown on tryptic broth was dependent on the length of time the broth had undergone digestion. The difference in this respect, however, seemed to be greater than could be explained by the further splitting of the polypeptids in the 4 weeks' fluid.

In other experiments evidence was obtained that when B. coli was grown under unfavourable circumstances, such as the absence of O or the presence of any cytolysing agent, the amount of heat liberated was greatly increased. In this respect resembling the Echinoderm egg, where Myerhof has shown (1) the amount of heat liberated in the unfertilised egg is greatly increased if the egg membrane has undergone cytolysis.

A great deal of variation was found in the amount of heat liberated by B. coli on different samples of tryptic digest. This was probably due to the fact that no attempt was made to standardise their content of amino-acids. The lowest amount of heat was obtained from one lot of broth that had been digested for an exceptionally long time (7–8 weeks) and had then been stored in the laboratory for almost a year. This

broth (about 5 litres) served for a large number of experiments and always gave the same result no matter how the experiments were varied. The results of the experiments described under 1 and 2 were obtained with it. None of the broths prepared subsequently gave such good results but their digestion was never carried so far or so thoroughly as in the first instance. The exclusion from these broths of any carbohydrates was confirmed by careful tests. The 1 week's digest was appreciably richer in tryptophane than the 4 weeks' digest, and its total solids somewhat greater, otherwise they seemed much the same.

In the present paper five typical experiments are described from among a large number carried out, which give a fair idea of the results obtained. It is hoped in future experiments where the amino-acid content of the broths will be standardised, more uniform figures will be attained.

Technique. The method used in making the heat estimations has been described by A. V. Hill(2). In the following experiments it has been modified in several minor points. It is based on the measurement of the relative temperature change taking place between the contents of two or more Dewar vacuum flasks, one of which contains water serving as a control, while the others contain the solutions whose heat production is to be measured. It has been shown by Hill, that slight alterations in the volume of the fluid contents of these flasks alters very considerably their rate of temperature fall. Thus by decreasing or increasing its contents, a flask can be made to have any desired rate of temperature fall. If an appropriate quantity of fluid is placed in each flask, any number of flasks can be given the same rate of temperature fall, that is the same rate of conduction of heat to the outside. They can then be employed in making a differential heat determination, provided they are uniformly exposed to the same external temperature variations. Thus errors due to external causes are almost entirely eliminated, and it is possible to carry out accurate heat determinations without being forced to use the complicated thermostats which are otherwise required. Hill has shown that it is possible to estimate to within 3 p.c. of its value, the liberation of 1 gram calorie per gram of flask contents in 10 hours, and in many of my own measurements I have been able to attain this degree of accuracy.

The ordinary narrow-necked silvered Dewar flasks were employed. These are made in two sizes as "refills" for commercial thermos bottles. The small size have a capacity of a little over 400 c.c., while the large hold slightly over 800 c.c. of fluid. On account of their greater volume, the larger flasks possess a coefficient of heat loss very considerably less

than that of the smaller, and for this reason are a little more accurate for making determinations, although their larger volume requires a much greater amount of culture fluid. I am indebted to Prof. Hill for placing at my disposal two 400 c.c. flasks, which were exceptional for having almost identical coefficients of heat loss. They could be employed differentially by placing the same amount of fluid in each. They will be referred to as flasks *R* and *L*. Their coefficient of heat loss when submerged in the water-bath at 37° C., was 0·0506. The greater part of this heat loss was due to conduction through the thick rubber stopper with which the flasks were closed when submerged in the bath. The large flasks used in these experiments (Exp. 3) and numbered 3, 4 and 5, when containing 790, 810 and 654 c.c. of fluid respectively, had almost identical coefficients of heat loss, *i.e.* $k = 0{\cdot}0403$.

The flasks were mounted in open wire-work baskets and arranged to clamp on the bath so that they were completely submerged in the water of the bath. Its temperature was kept at 37° C. by means of a thermo-regulator. The water in the thermostat was kept stirred by bubbling compressed air through it from a number of fine air-jets distributed evenly over the bottom of the bath. This stirring was sufficiently effective to render it impossible with a Beckmann thermometer to distinguish much more than a hundredth of a degree Centigrade between any two points in the water of the bath.

A copper constantan thermo-couple was connected to a sensitive Ayrton-Mather galvanometer with one junction in each flask, so any deflection of the mirror gave the difference of temperature between the two flasks. The sensitivity of the galvanometer was such that at a distance of 3·5 metres each millimetre on the scale represented ·00139° C. The flasks when submerged in the thermostat were closed by thick rubber stoppers. Through these, holes were drilled into which a short length of small glass tubing was inserted, which projected slightly above the upper surface of the cork, on this a small piece of rubber tubing was attached, which was long enough to reach well above the surface of the water when the stopper was in place in the flask, and the flask itself sunk down in the water of the bath, and down this tubing and through the cork the junction of the thermo-couple was inserted. These tubes served therefore the double purpose of introducing the thermo-couple junction, and at the same time of allowing any gases formed during the experiment to escape from the flask, without forcing out the cork.

The leads from the thermo-couples were brought to a specially constructed dial box furnished with a revolving key by means of which all

the thermo-couples could be put in circuit with the galvanometer in turn. It was arranged so that if this key pointed to the first figure on the dial, all the thermo-couples were short-circuited through the box, thus avoiding an injury to the galvanometer during the preliminary adjustment of the thermo-couples at the commencement of an experiment. A second key on the box, served to throw a number of resistances into the circuit at any time required. The wires of the leads, and all terminals, including those of the galvanometer, were made of copper throughout, thus eliminating any possible disturbing external thermo-electric effects.

The flasks after having their coefficients of heat loss determined in the manner described by Hill(1), were finally calibrated directly by means of the thermo-couples and the galvanometer. This method always gave a slightly smaller value for k, than that obtained by the use of the formula $\frac{T-T_0}{A-T_0}e^{-kt}$. In many instances, however, the two methods gave similar values for k. I have always preferred to use the figure given by the second method.

The procedure in carrying out one of the final calibrations was usually as follows. The proper amount of water was placed in a pair of flasks to give them similar values of k, this amount of water having been determined by the above-mentioned formula. One flask was then carefully adjusted with a Beckmann thermometer to have exactly the same temperature as the thermostat, which was 37° C., and trouble was taken to see that this control flask did not differ from the bath temperature by more than a hundredth of a degree C. The second flask (which was the one actually under calibration) was given a temperature half a degree C. above the first, both were then submerged in the water of the bath after having their stoppers and thermo-couple junctions placed in position. They were allowed to stand in the bath for an hour to warm up and were then raised till their necks were above the surface of the water, opened and their temperature readjusted finally. They were then resunk in the bath and after 30 or 40 minutes a series of readings were taken with the galvanometer extending over a period of three or four hours. These readings were then plotted out and k determined from the curve. The temperature of the thermostat was meanwhile carefully watched to see that it had not varied. To equalise the temperature throughout the contents of the flask it was gently shaken while in the bath a short time before each reading. The same method of stirring was also followed in making experiments as it was found impossible to stir the flask contents by the method recommended by Hill on account of the foaming

and frothing which takes place when air is bubbled through tryptic broth. Moreover the high temperature (37° C.) at which the experiments were carried out, made it impossible to use air on account of the evaporation and cooling which would have taken place.

To measure the actual heat production during any particular period of time, it is necessary to measure the increase of temperature of one flask over that of another at the end of the period, and multiply this by the capacity of the flask. In these experiments the capacity of the small flasks was 400 plus a correction of 20 for the material of the flask, giving a total of 420. In those experiments where the large flasks were employed the capacity was double this figure. If no heat were lost by conduction to the outside this product would represent the total heat liberated, but the Dewar flask in which the heat is being measured loses heat continuously, it is necessary that this heat loss be determined and added to the above value. The temperature fall during the period is the sum of all the falls of temperature at each instant. The rate of fall at any instant is measured by $k(T - T_1)$, where k is the coefficient of temperature loss and $(T - T_1)$ the difference of temperature between the two flasks at that instant. The total temperature fall is thus obtained by integrating $k(T - T_1)$ with respect to time, and this value is accurately obtained by measuring the area of a curve, plotting temperature differences (represented in galvanometer scale readings) against time.

Thus the total heat produced is equal to the capacity multiplied by the final temperature difference between the flasks plus this value just obtained, that is k (area of curve).

The heat production.

Exp. 1 (*a*). A quantity of emulsion of B. coli (24 hours culture) in saline solution (representing about ·1 of a gram of dried bacteria) was added to a mixture of peptone (1 p.c.) and glucose (about 4·5 p.c.). 2 grams of chalk were added, with a view to neutralising the acid formed during fermentation. After warming to 37° C. and thoroughly shaking, 400 c.c. of the mixture was introduced into flask *L*, and growth allowed to take place for 24 hours, the heat developed being measured at intervals. The exact quantity of the glucose introduced in 400 c.c. of fluid was found to be 16·8 grams. At the end of the experiment this had diminished to 11·6 grams. Thus 5·2 g. had been fermented. The heat produced during this period of time was 311·5 gram calories for the 400 c.c. of flask contents.

In this experiment about 1·6 p.c. of the energy contained in the

glucose was liberated as heat, but the whole of this heat was not produced from the glucose, for the living bacteria produce heat from the peptone solution in the absence of any sugar.

If we turn this heat value and growth weight into gram calories of heat produced per c.c. of flask contents we obtain the following value: The glucose peptone broth liberated in 22 hours, ·780 g. cals. per c.c. of flask contents.

Exp. 1 (*b*). A similar quantity (·1 gram) of the same culture of B. coli as that used in the first part of this experiment was added to 400 c.c. of Cole and Onslow's tryptic broth(3) and placed in flask *L*; flask *R* being used as a control with 400 c.c. water. The tryptic broth used was that mentioned on p. 50 as having been digested for an exceptionally long time. The stock broth had been diluted three times with an equal bulk of saline solution and its *p*.H adjusted to 7·4. The contents of the flasks were then warmed to the temperature of the water bath, the thermo-couples and corks placed in position and both flasks completely submerged in the water as described in the previous section.

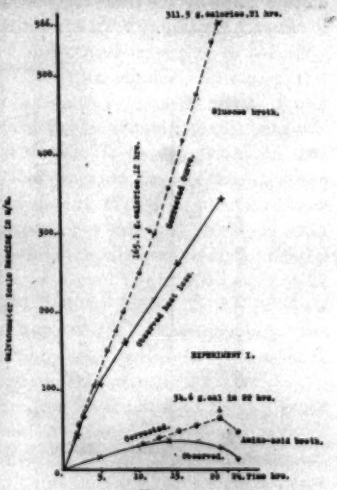

Fig. 1.

Fig. 1 shows the galvanometer scale readings over a period of 24 hours for this culture, and the curve corrected for heat loss shows that it gave off 34·6 gram calories of heat for the 400 c.c. of flask contents. The amount of growth that had taken place in the culture during this period, was determined by rough counts on the number of bacteria. The number in this culture was about the same as that of the previous glucose experiment. If anything there was a slightly greater growth in the amino-acid culture.

If we reduce the result of this experiment to values of gram calories per c.c. of flask contents, we obtain the following value: The amino-acid culture gave off in 22 hours ·085 g. cal. per c.c. of flask contents.

The previous glucose culture for the same amount of growth, gave

off, as said above, ·780 g. cal. per c.c. of flask contents, *i.e.* 8·5 times the amount of heat given off by the amino-acid culture.

In order however to compare with any degree of accuracy the magnitude of the process of growth with that of the resulting heat production, we need to know the weight of the bacteria concerned. In the subsequent experiments the bacteria were weighed after drying *in vacuo* at 37° C. This method has some drawbacks, but on the whole proved the most satisfactory. In some instances it was controlled by planting out the culture fluids by the three layer method and counting colonies.

Exp. 2 (a) (Fig. 2). To a solution of peptone and glucose (peptone

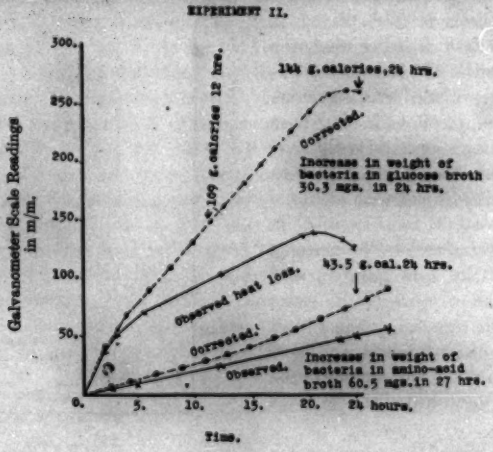

Fig. 2.

1 p.c. and glucose ·5 p.c.) was added 9·6 mg. bacteria (dried weight). The volume of the solution as before was 400 c.c., flask *L* being used for the culture fluid, while flask *R* was employed as the control with water. The experiment was continued for 24 hours, at the end of which time 144 g. cals. has been liberated. The weight of sugar which had disappeared from the solution as determined by reduction was ·99 or 1 gram approximately. While this experiment was in progress, the remainder of the broth used to inoculate the flask, was centrifuged and the weight of the bacteria in the deposit determined after drying *in vacuo* at 37° C. At the end of the experiment, the whole of the fermenting fluid in the Dewar

flask was likewise centrifuged, and the weight of bacteria determined again after drying. The weight of bacteria introduced at the beginning of the experiment was as already stated 9·6 mg. at the end of the experiment it was 40 mg. Thus considerable growth had taken place, and this had brought about the fermentation of fifty times its weight of glucose in 24 hours. If we turn these figures as before, into gram calories of heat produced per c.c. of flask contents per mg. of growth obtained in 24 hours, we have the glucose peptone broth producing ·0120 gram calorie per c.c. flask contents per mg. of growth.

Exp. 2 (*b*). One Roux bottle of a 13 hour growth on nutrient agar of B. coli was emulsified in 200 c.c. of amino-acid belonging to the same lot of digest as that used in the previous experiment. 25 c.c. of this emulsion was added to 375 c.c. similar broth contained in flask *L* and then was warmed to 37° C. The culture was maintained at this temperature in the thermostat as described in the previous experiments. The total heat produced in 24 hours was 43·5 gram calories. At the beginning of the experiment the weight of bacteria introduced into the flask was 9·5 mg., and at the end of the experiment this had increased after 27 hours to 70 mg. (in each case as before the numbers refer to material dried *in vacuo* at 37° C.). Thus in this culture 1 mg. growth gave ·0017 gram calorie in 24 hours[1] per c.c. of flask contents. In the previous portion of this experiment, the glucose gave ·0120 gram calories for the same amount of growth, thus the heat liberated by the glucose was seven times the amount produced by the amino-acid culture.

Exp. 3 (Fig. 3). In this experiment a fresh batch of tryptic broth was employed; this broth had only been digested a week and gave off a much larger amount of heat than that used in the two previous experiments. To 765 c.c. glucose (1·5 p.c.), peptone (1 p.c.) 25 c.c. of 24 hours culture of B. coli was added. This was shaken up and placed in flask No. 3 (large size). In flask No. 4, 810 c.c. water was placed as a control. In flask No. 5, 629 c.c. 1 week's digested tryptic broth was added. This broth had been diluted twice. To this flask 25 c.c. of the same emulsion of B. coli as that used for the glucose flask was added bringing its total contents to 654 c.c. Two thermo-couples were arranged to have one junction each in the control flask while the other junctions were in one case in the glucose culture and the other in the amino-acid flask. The contents of the flask were all warmed up to the temperature of the thermostat and the flasks sunk in the water of the bath. The experiment

[1] The weight was determined after 27 hours not 24, so the value given is not quite accurate; the error however is hardly enough to alter the value given above appreciably.

was continued for 24 hours and Fig. 3 shows the galvanometer scale readings with the corrected curve for heat loss over this period.

It was found that at the beginning of the experiment ·0154 mg. bacteria (dried) had been added to each flask. In the case of the glucose peptone culture, this had increased to ·0356 mg. and in this time the culture had given off 285 gram calories for 790 c.c. of flask contents. In the case of the tryptic digest this had produced in the same time ·0536 mg. growth, and given off 146 gram calories for 654 c.c. of flask contents.

If we reduce these values to gram calories per mg. of growth per c.c.

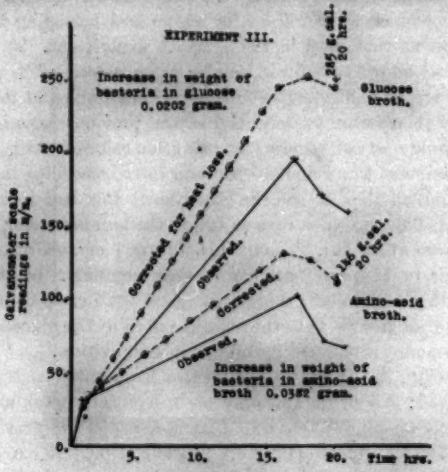

Fig. 3.

of flask contents as before, we find the glucose broth gave off ·0185 g. cal., while the amino-acid culture gave off ·0059 g. cal. per mg. growth per c.c. The glucose broth in this experiment gave off three times more heat than the tryptic digest for similar quantities of growth. Thus while in the first two experiments the tryptic broth gave off 7–8 times less heat than the glucose in this last, it only gave off three times less heat.

Exp. 4. In the experiment the amount of heat given off by the growth of B. coli on 1 week's tryptic digest was compared with that given off by the growth of the same organism on a 3 weeks' digest. Two litres of a 1 week's casein digest was divided in two equal portions, one

of which was immediately filtered, diluted three times with an equal bulk of saline solution, its p. H adjusted, sterilised and stored in the cold. The second portion, was digested for an additional 2 weeks, filtered, diluted in the same way, and its p. H adjusted like the first portion, to 7·4. The large flasks were used. An equal quantity of the same emulsion B. coli was added to both flasks (about ·015 mg.). The experiment was continued for 22 hours; in this time the weight of bacteria in the 1 week's digest had increased to ·2208 mg., while in the 3 weeks' digest it had increased to ·2720 mg., while the 1 week's digest gave off 704 g. cals., and the 3 weeks' digest 478 g. cals., for these amounts of growth respectively. In the case of the 1 week's digest, this works out at ·0054 g. cal. per mg. growth per c.c. flask contents in 22 hours, while in the 3 weeks' digest, this figure was ·0027 g. cal. per mg. per c.c. in the same time. Thus the amount of heat given off by B. coli when grown on 1 week's tryptic digest was almost twice that given off by the same organism on 3 weeks' digest, for the same amount of growth. This experiment was repeated three times with very similar results.

Exp. 5. In this experiment it was sought to determine the effect of the absence of oxygen on the amount of heat produced by B. coli when grown in glucose peptone broth. 400 c.c. glucose peptone broth (0·25 each) was placed in flask *R*, and a similar quantity in flask *L*. To each flask ·08 mg. B. coli was added. One flask was in free communication with the air, while the other (*R*) was rendered anaerobic by boiling the fluid under diminished pressure and replacing the air by nitrogen. The experiment was continued for 24 hours; at the end of this time about the same amount of glucose had disappeared from each culture, while the total dried weight of bacteria in each flask had not altered, planting out some of the fluid from each flask showed however that there were four times as many organisms alive in the aerobic culture than in the anaerobic, although this culture had given off 230 gram calories of heat in excess of the aerobic. The unhealthy bacteria of the anaerobic culture in spite of their fewer numbers, had given off considerably more heat than the healthy, in breaking down the same amount of glucose. Thus the normal process of metabolism would seem to be a far more economical one than the pathological.

A number of experiments were carried out in which various cytolysing agents were added to the culture fluids, such as bile salts, butyric acid, etc., growth of B. coli under these conditions almost always gave a greater amount of heat than under normal conditions for the same amount of growth.

Conclusions.

1. When B. coli is supplied with abundant free amino-acids already prepared, the process of building these into the living protoplasm is an extremely economical one, in which little energy is wasted. While different batches of tryptic digest gave widely divergent results in this respect (probably due to the fact that their amino-acid content was also very different), as compared with the growth of the same organism on glucose peptone broth, the process is from 3–8 times more effective, although in the fermentation of glucose this germ would seem to use surprisingly little (about 1 p.c.) of the total energy available in the carbohydrate molecule.

2. Tryptic broth that has been digested a short time (1 week) produces considerably more heat with B. coli than the same broth digested for a long time (3–8 weeks). This difference would seem to be far too great to be accounted for by the extra hydrolysis the polypeptids had undergone in the 3–8 weeks' fluid.

3. The pathological process of metabolism either with tryptic digest or any other culture fluid, is always accompanied by a greater liberation of heat than is given by the same amount of growth on the same medium under normal healthy conditions, so that pathological is less economical than normal growth.

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THE PHYSIOLOGICAL CHANGES PRODUCED BY RADIUM RAYS AND ULTRA-VIOLET LIGHT IN THE EGG OF NEREIS.

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Exposure of the unfertilised eggs of *Nereis limbata* to the β- and γ-rays of radium brings about certain very definite changes in the process of membrane formation and jelly secretion, which ensues when the eggs are subsequently fertilised (1). In the present paper three questions are examined with respect to this reaction.

(i) What is the effect of other kinds of radiation on this process?

(ii) What is the nature of the change produced in the egg by radiation?

(iii) How may the specific differences in the effects of different radiations be explained?

I. Comparison of different kinds of Radiation.

β- and γ-rays. The cortical changes which occur when fertilisation takes place have been described by Lillie (2). The surface of the unfertilised Nereis egg is covered by a thin, yet clearly visible, *vitelline membrane*. Beneath this is a coarse layer of alveoli, in which is held the jelly which is to be secreted upon fertilisation. When the sperm becomes attached to the ovum, the jelly is extruded through the vitelline membrane, takes up water, and forms a thick layer outside of the egg. The alveoli, which thus have been emptied of their contents, become filled with water, with the result that a thin transparent *perivitelline space* separates the vitelline membrane from the underlying protoplasm.

In eggs exposed to β- or γ-rays no change is observed before insemination takes place, but the result of the process of jelly secretion and the formation of the perivitelline space beneath the vitelline membrane is abnormal in two fundamental respects. The volume of jelly which is formed outside the egg is reduced, and the volume of the perivitelline space is increased (Fig. 1). No change can be detected in the appearance of the vitelline membrane itself.

Fig. 1.

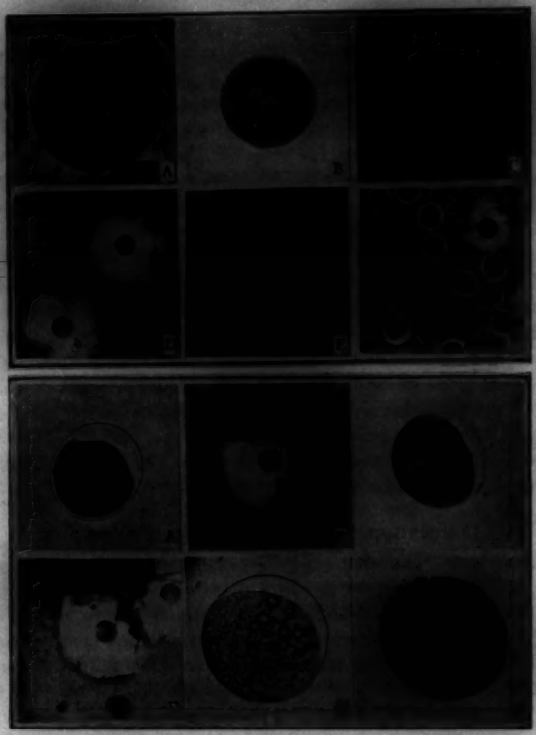

Fig. 2.

Fig. 1. Photomicrographs of Nereis eggs. *A*. Unfertilised—slightly compressed, showing alveolar layer. *B*. Unradiated fertilised egg showing normal perivitelline space. *C*. Radiated with β-rays; perivitelline space $14{\cdot}3 \times 10^3\ \mu^3$. *D*. Unradiated eggs in suspension of india ink in sea-water, showing jelly. *E*. Radiated with β-rays. *F*. Illustrating maximal effect of β-rays.

Fig. 2. Photomicrographs of Nereis eggs. *A*. Radiated with α-rays. *B*. In suspension of ink showing effect of α-rays on jelly. *C*. Radiated with light from carbon arc. *D*. The same, showing effect on jelly. *E*. Radiated with Schumann rays—photographed with visible light. *F*. The same, photographed with light of 2800 Å.U.

α-rays. Radium emanation, contained in a small glass bulb, the wall of which was sufficiently thin to allow the escape of α-rays, was used as a source of radiation. The Nereis eggs were exposed to the α-radiation in drops of sea-water hanging from a glass slide above the bulb. Since the eggs sank to the bottom of the drop α-rays entered them without passing through an appreciable thickness of sea-water. The loss of energy of the α-rays before reaching the eggs was due solely to passage through the walls of the bulb and the intervening air.

When the unfertilised Nereis eggs are exposed to α-rays no change can be observed in their appearance until they are fertilised, unless the intensity of radiation is excessive when they are rapidly cytolised. After fertilisation, membrane formation and jelly secretion occur, the perivitelline space being abnormally thickened and the volume of the secreted jelly reduced. A striking difference distinguishes such eggs from those which have been exposed to β-rays alone. Whereas the effect of the β-rays is a symmetrical one, jelly secretion being reduced and thickness of the perivitelline space increased uniformly on all sides of the eggs, the change in the eggs exposed to α-rays is much greater on one side than the other (Figs. 2 *A* and *B*).

Sunlight. Unfertile Nereis eggs exposed to bright sunlight at mid-day in August for periods up to thirty minutes exhibit upon being fertilised, cortical changes in every way like those occurring in control eggs kept in the dull light of the laboratory. The light of the visible spectrum together with such ultra-violet light as is present in sunlight does not appear to affect the Nereis eggs in the way characteristic of radium radiations. This result indicates that light waves between 7000 and 3000 Å.U. are ineffective.

The carbon arc and the quartz mercury vapour lamp. In order to test the effects of ultra-violet light of shorter wave lengths than those occurring in sunlight, eggs were exposed to the light from a small carbon arc and from a quartz mercury vapour lamp. The unfertile eggs were placed under the light source in a shallow layer of sea-water in a vessel surrounded with ice to prevent heating. Twenty minutes' exposure to the carbon arc at a distance of 3 or 4 inches caused the eggs to develop on being fertilised; a perivitelline space widened on one side and accompanied by a reduction in the jelly on the same side (Fig. 2 *C* and *D*). Similar effects were produced by the quartz mercury vapour lamp, except that exposures as short as 2·5 minutes sufficed. If a thin microscopic cover glass was introduced between the eggs and the carbon arc, exposure to the light no longer caused any abnormality in the fertilisation mem-

brane. It may be concluded therefore that the effective light is of a wave length shorter than 3100 A.U., which marks the limit of transmission of crown glass. The ultra-violet light emitted by the carbon arc extends to wave lengths as short as 2200 Å.U., that of the quartz mercury vapour lamp to about 1800 Å.U. We can place the wave length at which electromagnetic waves become effective in producing the reaction somewhere between 3000 and 2200 Å.U.

Schumann rays. Light of the very short wave lengths of the Schumann region was obtained from the hydrogen discharge tube described by Bovie(3). The rays from this instrument which are most destructive to protoplasm were shown by Bovie to be shorter than 1700 Å.U. The shortest waves which can escape through the fluorite window of the discharge tube are 1250 Å.U. When Nereis eggs were placed directly on the fluorite window, exposed to radiation for 15 seconds or longer and then fertilised, subsequent examination showed that the perivitelline space was greatly thickened on one side, but of normal dimensions on the other (Fig. 2*E*). It appears from this that the physiological effect produced by the shorter wave lengths of the light from the carbon arc and the quartz mercury vapour lamp is duplicated by the very short waves of the Schumann region of the spectrum[1].

II. Analysis of the change produced by Radiation.

The similarity in the effects of the three kinds of radium radiation and of the shorter wave lengths of light suggests that some common mechanism underlies their action. Before we are justified in suspecting that this is the case we must enquire more closely into the nature of the physiological change which produces the phenomenon which is observed. The simpler this change is found to be, the less will be the probability that the common end effect produced by different kinds of radiation is due to physical actions of qualitatively distinct sorts.

Since changes in the volume of the jelly and the perivitelline space are reciprocals of one another, it is natural to suspect that in radiated eggs a certain quantity of the jelly substance is permanently retained within the alveoli and consequently serves to increase the volume of the perivitelline space. Such a retention would at the same time account for the decreased volume of the secreted jelly. This assumption was made

[1] We are indebted to Dr William Duane and to Dr Robert B. Greenough, Director of the Huntington Memorial Hospital, for placing radium emanation at our disposal, to Dr Bovie for the use of his hydrogen discharge tube, and to Miss Alice Klein for assistance in our experiments with it and for making the photographs shown in Fig. 2*E* and *F*.

by Packard(4) and our observations justify its unqualified acceptance as the considerations which follow will show.

(1) An increase in the volume of the perivitelline space does not follow the radiation of eggs, such as those of the mollusc, Cummingia, the echinoderms, Abracia and Asterias, and the Annelid, Chaetopterus, which do not normally secrete jelly at the time of insemination.

(2) When the action of rays is limited to one part of the egg the

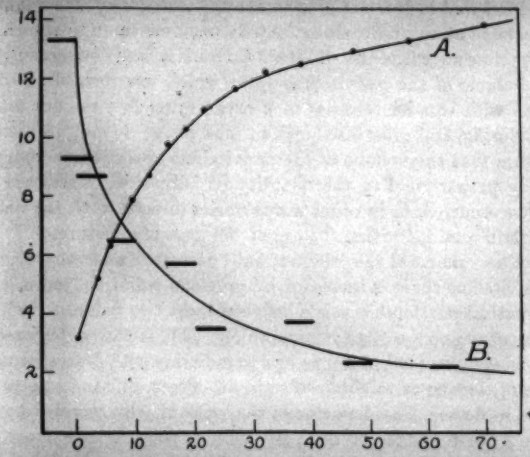

Fig. 3. The relation between the amount of jelly secreted at the time of radiation and the resulting effect on the volume of the membrane. Time elapsing after fertilisation measured in minutes along the abscissa. *A*. The diameter of the jelly secreted by normal eggs, measured in arbitrary units along the ordinate. *B*. The volume of the membrane resulting from radiation with 95 millicurie centimetres of β-rays for 5 minutes at various intervals before and after fertilisation.

The heavy horizontal lines indicate by their length the duration of radiation, and by their distance from the abscissa the volume of the resulting membrane, measured in 100,000 cubic microns along the ordinate.

volume of the perivitelline space is increased over this part alone and over the same part the volume of the jelly is found to be greatly reduced. This condition is produced in eggs exposed to α-rays, or to ultra-violet light (Fig. 2).

(3) The enlargement of the perivitelline space does not occur if the eggs are radiated after the jelly is secreted. The secretion of the jelly is not an instantaneous process, but goes on during a considerable period

of time after fertilisation occurs. By radiating eggs for short intervals at various times before and after insemination, it may be shown that the resulting volume of the perivitelline space decreases as the quantity of jelly which surrounds the egg increases. This fact is illustrated by Fig. 3, in which curve *A* is based on measurements of the diameter of the mass of jelly at various times after insemination. This curve may be taken as an indication of the course of secretion of jelly, but it must be pointed out that it does not give an absolute measure of the quantity of jelly secreted at any time, since the jelly must continue to absorb water for some time after it is first secreted. Curve *B* is based on measurements of the volume of the perivitelline space which was formed about eggs radiated with equal intensities of β-rays during five minute intervals before, during, and after insemination took place. It may be seen from this figure that the volume of the perivitelline space is in a rough way inversely proportional to the quantity of jelly already secreted when radiation occurred, or in other words varies directly with the quantity of jelly still retained within the egg at the time of exposure.

(4) The volume of the jelly not only decreases when the volume of the perivitelline space is increased by previous radiation, but a definite mathematical relationship exists between these two volumes even when the magnitude of the effect varies widely. This fact was demonstrated by exposing several lots of Nereis eggs to radiation with β-rays for various periods of time so as to obtain a series of effects differing as greatly as possible in degree. The diameter of the mass of jelly secreted by these eggs and the thickness of the perivitelline space formed about them were measured, and from these data a calculation was made of the volume of the jelly and the volume of the perivitelline space formed by each lot. For the purpose of this calculation it was assumed that the eggs and their covering of jelly are spherical; the diameter of the egg, exclusive of membrane and jelly, was taken to be 128 μ and the necessary corrections were made for the volume within the jelly occupied by the egg and its more or less enlarged membrane. Table I contains the results of two experiments. It may be seen that as the period of radiation increases the volume of the perivitelline space also increases while the volume of the jelly decreases. A close parallelism exists between the increase in the volume of the perivitelline space and the decrease in the volume of jelly. The last column in this table contains the ratio between these values. The numbers expressing this ratio fluctuate at random about a mean value which is practically identical in the two experiments[1]. The

[1] The deviation of the individual values of this ratio is readily explained by the inac-

TABLE I. Nereis eggs exposed to β-rays at an intensity of 16·6 millicurie centimetres. Ten eggs in each lot. Measured perivitelline space 60 minutes after fertilisation, jelly 80 minutes after fertilisation.

Time of radiation Minutes	Vol. of perivitelline space $10^9 \mu^3$	Increase in perivitelline space $10^9 \mu^3$	Vol. of jelly $10^9 \mu^3$	Decrease in jelly $10^9 \mu^3$	Decrease in jelly / Increase in perivitelline space
0	1·30	—	1009·2	—	—
5	3·10	1·80	855·9	123·3	68·7
10	3·80	2·50	738·6	270·6	108·2
15	5·35	4·05	711·5	297·7	73·5
20	6·85	5·55	539·8	469·4	84·6
30	9·30	8·00	456·8	552·4	69·2
45	11·55	10·25	354·5	654·7	63·8
60	12·50	11·20	246·1	763·1	68·2
123	14·90	13·60	218·4	790·8	58·2
				Average	74·3

Intensity of β-rays 57·7 millicurie centimetres. Twenty-five to thirty eggs in each lot.

0	1·75	—	1039·8	—	—
5	6·10	4·25	629·6	410·2	96·5
10	7·70	5·95	518·0	521·8	67·8
15	10·50	8·75	355·5	684·3	65·1
20	13·35	11·60	321·0	718·8	62·0
30	15·00	13·25	179·8	860·0	64·8
45	13·35	11·60	165·8	874·0	75·3
60	13·50	11·75	137·6	902·2	76·8
90	12·60	10·85	62·2	977·6	90·0
				Average	74·8

simplest assumption that can be made in explanation of this relationship is that material which would normally be secreted to form jelly is retained, as the result of the radiation, within the perivitelline space and by taking up a certain quantity of fluid, brings about the enlargement of the perivitelline space. On the basis of this assumption the actual value of the constant ratio, which is about 75 in the experiments cited, must mean that each molecule of jelly substance together with the sea-water with which it becomes associated occupies a volume upon being secreted 75 times as great as does each molecule of this material which is retained within the vitelline membrane as the result of radiation.

An alternate hypothesis to explain the changes which radiation produces in jelly secretion and membrane formation might assume that all the jelly is secreted from the radiated eggs, but that its ability to take

curacies of the method of measurement. Fig. 1 shows that the jelly does not form a regular spherical mass as was assumed for calculation particularly in the case of the unradiated egg and that the limits of the jelly mass are not clearly defined. Consequently the measurement of the diameter of the jelly is subject to a considerable error, which is increased further by the calculations, for the volume of the jelly is a function of the cube of the diameter. If the error in measuring the diameter of the jelly amounts to 10 p.c., the error in the calculated volume will be about 30 p.c. As a matter of fact only two of the determinations deviate from the average by more than 25 p.c.

up water has been diminished as the result of some alteration in its physical properties, and that at the same time some other material within the perivitelline space undergoes a physical change in the opposite sense, with the result that it takes up large quantities of water and produces the swollen fertilisation membrane. We believe that this view is absolutely untenable for the reasons which follow:

(1) Eggs which are severely radiated do not secrete any jelly (Fig. 1 *F*). The effect must be due to a retention of this material rather than to an alteration in its ability to take up water after being secreted.

(2) This hypothesis does not explain why radiation becomes ineffective as soon as the jelly has been secreted from the egg. It would lead us to expect that the change in the perivitelline space might be produced at any time after fertilisation.

(3) On the basis of this hypothesis the close relationship which we have shown to exist between the magnitudes of the effect on the perivitelline space and the effect on the jelly must be assumed to be a chance coincidence.

The cause of the retention of the jelly by radiated eggs. Since we accept the hypothesis that the effects of radiation are due to the retention of a quantity of the jelly substance within the perivitelline space, we must enquire why this material fails to leave the radiated egg when fertilisation occurs. Two alternate forms of explanation present themselves.

1. Radiation may alter the physical properties of the jelly substance while it is within the unfertilised egg with the result that it is unable to pass through the retaining membranes when fertilisation sets up the process of jelly secretion.
2. Radiation may alter the physiological properties of the membranes of the egg with the result that they become less permeable to the jelly substance, and consequently interfere with its escape from the fertilised egg.

It may be noted that these possibilities are not mutually exclusive, and for all we can tell, both may actually occur. We believe, however, that the first hypothesis accounts for the greater part of the retention of the jelly, for reasons which are set down below[1].

[1] This view is rendered plausible also by the fact that the properties of the jelly, within the perivitelline space, is readily altered by a number of reagents. The quantity of water taken up can be shown to depend on the proportions of the salts in the sea-water, the hydrogen in concentration and the temperature. Many observations indicate that the different kinds of rays which we have found to cause the retention of jelly readily alter the physical properties of a variety of colloidal systems (Sekera, Kolloid Ztschr. 27. 145. 1920, and references there given).

(1) The jelly which is secreted by radiated eggs differs in its properties from that formed by normal eggs. This observation which was made by Packard(4) we have confirmed. The jelly of the radiated eggs does not have the usual sticky character which causes the normal eggs to tend to adhere to each other. As a consequence these eggs do not become matted together after the manner of the unradiated eggs when a number are caused to secrete their jelly in close proximity to one another. In the course of some microdissection studies which Dr Robert Chambers was kind enough to make for us it was observed that the jelly of the radiated eggs offered less resistance to the passage of the dissecting needle than did that of the unradiated controls.

(2) The ratio which exists between the increase in the volume of the membrane and the decrease in the volume of the jelly indicates, that as a result of the radiation the material which is retained has acquired properties in respect to its affinity for water quite different from the normal jelly substance.

(3) On the assumption that the properties of the jelly substance within the alveolar layer of the unfertilised egg are altered, it is possible to predict in mathematical terms the course of the reaction as it proceeds under uniform intensity of radiation. Such a prediction can be confirmed by direct experiment.

The amount of material undergoing change as the result of radiation at any instant of time will depend upon the intensity of radiation and the quantity of material left unchanged at that instant. If we express the quantity of jelly substance which is converted into the form retained within the perivitelline space by x, and the quantity of this substance present at the beginning of radiation as a, then the quantity left unchanged at any time, t, is expressed by $(a - x)$. The rate of transformation at any time will be

$$\frac{dx}{dt} = k(a - x)$$

where k is a constant, one factor of which depends upon the intensity of radiation. This is the formula for a unimolecular chemical reaction, and on integration yields the equation

$$\frac{1}{t} \log_{10} \frac{a}{a - x} = k.$$

The value of a can be found by direct observation. By determining the volume of the perivitelline space of eggs which have been so heavily radiated that no jelly is secreted by them and subtracting the volume of the normal space from this volume a figure is obtained which expresses the total quantity of jelly substance transformed by the radiation when

the reaction has gone to completion. This quantity is obviously equivalent to a. The value of x is given by subtracting the normal volume of the perivitelline space from the volume produced by radiation for any time, t.

In Fig. 4 the curve represents the theoretical course of the reaction, assuming k to equal 0·0125, in an experiment in which a was found to be $11{\cdot}65 \times 10^5 \mu^3$. The values of x determined experimentally lie as close to the curve as could be desired. We have confirmed the observation that the course of the reaction follows the monomolecular formula with a large number of experiments and believe this relationship to hold true

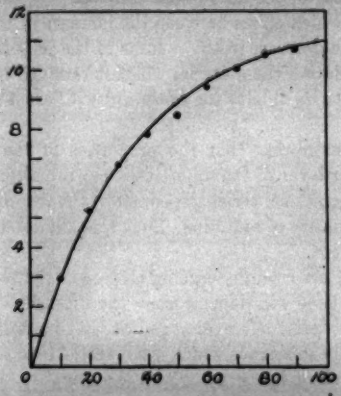

Fig. 4. Ordinate measures the increase in the volume of the perivitelline space, in 100,000 cubic microns. Abscissa measures time of radiation in minutes. Intensity of radiation, 15·7 millicurie centimetres.

at least for intensities of radiation of the magnitude employed in the experiment cited.

(4) If the jelly substance within the alveolar layer is altered by radiation, it should be possible to show in accordance with the law of Grotthus, that the effective kinds of rays are absorbed by this material. Obviously the α-, β- and γ-rays must be absorbed by the jelly substance, since the absorption of these rays is a function of the density of the matter through which they pass. The absorption of light waves on the other hand is determined by the molecular structure of the absorbing material. It is possible to carry out a critical experiment which demonstrates that the wave lengths which are effective are absorbed by the

jelly substance, and that this material is transparent to ineffective parts of the spectrum. Since the hypothesis assumes that the material which is acted on by the radiation is the same as that normally secreted to form the jelly, we have performed this experiment upon a preparation of jelly secreted by normal eggs.

The jelly substance of the Nereis eggs was obtained by the following procedure. Eggs from a large number of worms were washed with four changes of filtered sea-water. They were then placed in a mixture of equal parts of filtered sea-water and 0·6 molecular KCl, which caused them to secrete their jelly. Contamination of the jelly by the addition of sperm was thus avoided. The jelly was separated from the eggs by thorough centrifuging and a quantity of 95 p.c. alcohol added, as the result of which the jelly substance separated out as a stringy white precipitate. This precipitate was dried in a desiccator.

A little of the dried material was powdered, mixed with a few drops of distilled water, and spread on a thin quartz plate. The material took up water, with the result that the quartz plate became coated with a thin transparent layer of jelly. By interposing this plate between various sources of ultra-violet light and the quartz spectrograph photographs were obtained showing which wave lengths were absorbed by the jelly. For light sources arcs of aluminium, cadmium and carbon were employed. The spectrum of the aluminium arc contains lines representing wave lengths as short as 1854 Å.U. When photographed through the jelly the line of shortest wave length to appear is of 2140 Å.U. The spectrum of the cadmium arc contains a number of lines in about this region, of which a line of 2144 Å.U. is clearly visible, one of 2116 Å.U. is no longer to be seen. The spectra show that over a range of wave lengths between about 3600 Å.U. and the limit of complete absorption there is a partial absorption of the light by the jelly substance. In passing from the visible portion of the spectrum in which the jelly is perfectly transparent to the region of complete absorption the lines become progressively dimmer, indicating that a partial absorption occurs which is more complete the shorter the wave length. When comparison is made with the spectrum of light which is transmitted by glass it is seen that the jelly is somewhat more transparent to light of short wave length.

In comparing the wave lengths of light which affect the process of membrane formation and jelly secretion with the wave lengths which are absorbed by the jelly substance we find that the wave lengths which affect the physiological process, *i.e.* the part of the carbon arc absorbed by glass, falls within the range in which the jelly substance is a partial

absorber of their energy. Light of longer wave lengths than that absorbed by glass is ineffective in altering the cortical changes in the egg and is absorbed only to the slightest extent or not at all by the jelly substance. Light shorter than about 2140 Å.U. is completely absorbed by the jelly and in accordance with this the very short wave lengths of the Schumann region are effective. These correlations substantiate the view that the change in jelly secretion produced by radiation is due to an alteration in the properties of the jelly substance within the alveolar layer; since they satisfy the principle of Grotthus, that the effective radiation will be absorbed by the material which is altered by photochemical action.

We fail to find any reason for adhering to the alternate possibility, suggested by Packard(4), that the retention of the jelly substance by the radiated eggs is due to a change in the permeability of the egg membranes. Packard advanced in its support the observation made by Dr G. L. Kite, in the course of microdissections of radiated Nereis eggs, that the membranes of these eggs are softer than normal and can be punctured without difficulty. This evidence is unconvincing because we are aware of no reason to believe that the permeability of the membranes is a function of their mechanical strength. It would be more logical to argue, if such an assumption is made, that the weaker membrane of the radiated egg would be more permeable, rather than less permeable, to the jelly substance. If a change in permeability occurs the jelly secretion would be expected to take place more slowly in the radiated egg. We have observed that the average time elapsing between the addition of sperm to a drop of sea-water containing normal Nereis eggs and the first appearance of the jelly, as shown by the pushing of suspended india ink away from the surface of the egg was 30·7 sec. in the case of fourteen measurements. The corresponding time in the case of fourteen eggs which had been radiated sufficiently to reduce the diameter of the secreted jelly to one half its normal value was 32·8 seconds. The difference between these two determinations, 2·1 seconds, is certainly within the limits of error in such observations and is not significant. In consideration of the satisfactory way in which the assumption that the jelly substance is altered by radiation explains the quantitative relations observed, and the positive evidence that its properties are altered it is justifiable to conclude that any change which may occur in the permeability of the membranes is negligible as a cause of the retention of this material within the perivitelline space.

III. Explanation of the specific effects of different Radiations.

It has been pointed out recently by Bovie(5) that rays from widely separated regions of the spectrum and from radium produce similar physiological effects, provided that their ability to penetrate the organism is such that similar parts are radiated. This generalisation suggests the existence of a fundamental similarity between the mechanisms underlying the action of the different kinds of radiation upon living matter and indicates that apparent specific differences in the effects produced by particular types of radiation are attributable solely to differences in the degree and place of absorption of radiant energy. The data on which this generalisation is based are the malformation of embryos, the premature differentiation of tissues, the activation of unfertile eggs, and the acceleration and retardation of the rate of cell division and growth. These are complex phenomena which might be brought about by reagents acting through quite different mechanisms on different parts of the underlying processes. The disturbance in the cortical changes in the egg of Nereis is a more suitable process with which to test the validity of these inferences, since it seems to be a simple phenomenon which involves the alteration of the properties of a single substance within the egg, and which in our experience can be imitated by no reagents other than radiations.

The radiations may be grouped according to their effect on the Nereis egg into those which produce an equal change on all sides of the egg (β- and γ-rays), and those which cause an alteration limited chiefly to one side (α-rays and ultra-violet light). These differences would find a ready explanation if it could be shown that the radiations of the first group are absorbed to such a slight extent in passing through the egg that the energy of the emergent rays was practically as great as that of the incident rays, and that those of the second group are almost completely absorbed before traversing the thickness of the cell.

Absorption of β- and γ-rays. When it is considered that γ-rays are more penetrating than the hardest X-rays it is obvious that the energy lost by them in penetrating the Nereis egg is negligible in comparison to the initial energy of the radiation. The β-rays, in comparison, are much more readily absorbed by matter. We have shown (1) that the absorption of the physiologically effective β-rays by aluminium may be expressed by a coefficient of absorption of 23·9 cm.$^{-1}$. If elements of high atomic weight are not involved the absorption coefficient for β-rays is approximately inversely proportional to the density of the absorbing material(6).

Since the density of aluminium is 2·7, and that of the egg (approximately equal to sea-water) about 1·02, the coefficient of absorption of these β-rays in the egg is roughly $23·9 \times \frac{1·02}{2·7}$ or 9·0. From the absorption equation $\frac{I}{I_0} = e^{-\mu d}$ where I is the intensity of radiation after passing through a thickness of matter d, and I_0 the initial intensity, and μ the coefficient of absorption it may be estimated that the intensity of the β-rays would diminish only about 11 p.c. in traversing an egg ·0128 cm. in diameter. It is consequently to be expected that the effects of the β-rays will be nearly the same on all sides of the egg.

Absorption of α-rays. The absorption of α-rays by matter is proportional to the square root of the atomic weight of its constituents(6). Because of the high water content of protoplasm and the low atomic weight of most of its other components the stopping power of the Nereis egg cannot differ greatly from that of water. Michl(7) has found that the range of α-rays from polonium in water is 0·0032 cm. It may be estimated from this that the range of the most penetrating α-rays emitted by our source, those from radium C, would be 0·0059 cm. Correcting this figure for the determined stopping power of the wall of the bulb in which the emanation was confined and for the air intervening between it and the egg we obtain 0·0031 cm. as the maximum depth of penetration of the α-rays. This is about one-fourth the diameter of the egg.

Information can be obtained by direct observation which shows that the α-rays do not penetrate through the entire thickness of the cell and indicates approximately how far into the cell they do penetrate. The first point is shown by measuring the least thickness of the perivitelline space of eggs which have been exposed to the mixed α-, β- and γ-radiation from the bulb of radium emanation and comparing this effect with measurements of the width of this space in eggs which have been exposed for a similar time to the same radiation from which the α-rays have been filtered by a thin screen of aluminium.

The results of a number of such measurements are shown by a comparison of the pairs of figures in Table II, Column III, and indicate that the effect due to such rays as penetrate through the entire thickness of the egg is no greater than the effect produced by the β-rays and γ-rays alone. It may be concluded that α-rays do not penetrate the entire thickness of the protoplasm of the Nereis egg, a distance of 130 μ.

A rough estimate of how far the α-rays penetrate into the Nereis eggs can be made from measurements of photographs of eggs which have

TABLE II. Nereis eggs radiated at various distances from a bulb containing 8·8 millicuries of radium emanation. Measured 70 minutes after fertilisation. Fifteen to twenty eggs in each lot.

I	II	III	IV	V	VI
Distance	Maximal thickness	Minimal thickness	Intensity β-rays	Intensity α-rays	Intensity α-rays / Intensity β-rays
cms.	μ	μ	millicurie cms.	millicurie cms.	
·95	—	15·9 ±·28*	9·75	—	—
1·57	21·0 ±·53	11·9 ±·39 12·4 ±·16*	3·58	33·4	9·35
2·17	18·1 ±·38	10·4 ±·17 7·6 ±·11*	1·87	16·7	8·94
2·80	14·9 ±·53	6·97±·10 6·62±·10*	1·13	9·42	8·35
3·37	15·7 ±·78	5·84±·16	0·78	5·02	6·44
4·02	10·5 ±·85	4·78±·13 4·92±·07*	0·55	1·94	3·53
4·57	8·28±·49	4·46±·13	0·43	0·79	1·84
5·17	6·34±·43	4·81±·12 4·11±·13*	0·33	0·44?	1·33?
5·80	3·68±·26	3·05±·15	0·26	0·35?	1·35?
6·37	6·80±·30	5·49±·13 4·67±·15*	0·22	0·31?	1·41?
7·00	3·90±·25	3·22±·17	0·18	—	—
7·60	5·95±·38	3·72±·11 2·83±·12*	·015	—	—

Figures marked * obtained by radiating through aluminium filter 0·0102 cm. thick.

been subjected to heavy α-radiation. Such photographs show that the perivitelline space covering the surface of the egg on one side is of uniform width and not greatly thickened. A meridian can be drawn on such eggs which divides this area from the surface over which the vitelline space is greatly thickened. The plane of this meridian must mark quite closely the limit beyond which the α-rays fail to penetrate. Although the distortion of the protoplasm on the side covered by the enlarged membrane offers an obstacle to the accurate determination of the depth of penetration by this method, it may be concluded from measurements of a number of photographs that the plane beyond which no α-rays pass is about 0·0065 cm. from the side of incidence.

These figures refer to the absorption by the egg of only the most penetrating α-rays. Most of the rays reaching the egg have already lost much of their energy and are stopped much sooner by the protoplasm. This is because only those rays which pass radially through the wall of the glass bulb will possess a maximum energy on emerging, while those rays which pass obliquely through its wall will have their penetrating power reduced in proportion to the length of their path through the glass. The solution of our problem requires a knowledge of the quantity of energy given off by the α-rays in each part of the cell traversed by

the radiation, and consequently some indication is demanded of the intensity of the rays reaching different depths of the cell.

A careful physical investigation would be necessary to determine the distribution of the α-rays from the bulb with respect to penetrating power. Although this has not been made, we have been able to estimate in a rough way from the data contained in Table II the relative intensity of the physiologically effective α- and β-rays at different distances from

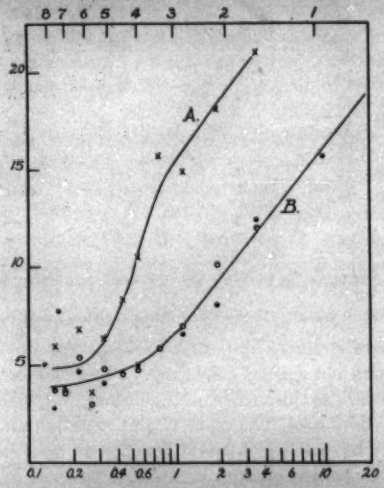

Fig. 5. Graph based on data in Table II, showing relation of thickness of membranes produced by α-rays (curve *A*) and by β-rays (curve *B*) at various distances from bulb. Thickness of membrane measured in microns along ordinate; intensity of β-rays measured in millicurie centimetres along lower abscissa; corresponding distances measured in centimetres along upper abscissa.

the bulb. Since the loss in energy of the β-ray in passing through a few centimetres of air is negligible, the change in the ratio of the intensity of the α-rays to that of the β-rays with increasing distance gives a measure of the loss of intensity of the α-radiation due to its passage through air. Assuming that the intensity of the β-rays is expressed by the reciprocal of the square of the distance, Curve *B* has been plotted in Fig. 5 from the data in Columns III and IV. It relates the thickness of the perivitelline space and the intensity of the β-radiation producing it.

Curve *A*, plotted from the data in Column II indicates the thickness of the space produced by the combined α- and β-radiation at corresponding distances[1]. By comparing these two curves the intensity of β-radiation required to produce a perivitelline space as thick as that produced by the combined α- and β-radiation at any given distance may be found and taken as a measure of the intensity of the combined radiation at that distance. The increment due to β-rays alone at that distance may be also evaluated from Curve *B* and by subtracting this from the intensity

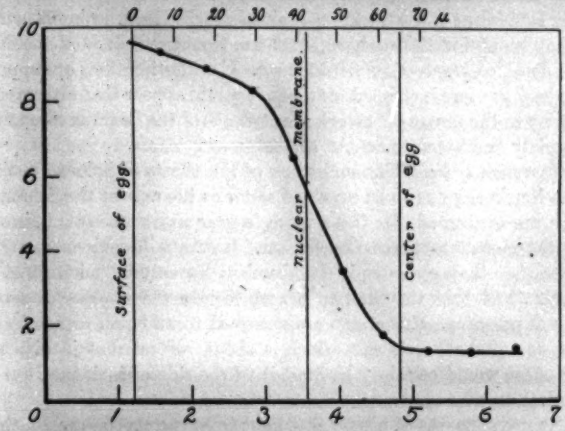

Fig. 6. Ordinate measures strength of beam of α-rays in arbitrary units.
Abscissa measures depth of penetration in centimetres of air (lower scale); in microns of protoplasm (upper scale).

of the combined α- and β-radiation a value is obtained representing the intensity of the α-radiation alone. Such values are contained in

[1] There is some difficulty in measuring the α-ray effect. The greatest thickness of the perivitelline space on the side of incidence must be determined. Relatively few eggs will be in a position to allow this to be done, and some judgment is required in selecting those which are lying with the axis along which the incident radiation travelled perpendicular to the optical axis of the microscope. Any error in selecting makes the measurement too small. Further, many eggs which have not been exposed to α-rays exhibit slight inequalities in the thickness of their membranes and the purposeful selection of these indicates an α-ray effect which does not exist. In plotting the intensities we have chosen a logarithmic scale because we have found (1) that throughout a considerable range the thickness of the perivitelline space varies directly with the logarithm of the intensity of the β-radiation, and the curve consequently becomes a straight line.

Column v. Column vi contains the ratio of the intensity of the α- and β-rays from each distance, and this ratio represents the strength of a pencil of α-rays at different distances from the bulb[1]. Fig. 6 represents the decline in strength of the α-rays in their passage through air.

If we assume that the stopping power of all parts of the egg is the same this curve may be taken to represent the relative strength of a beam of α-rays as measured by its ability to effect protoplasmic changes as it passes into the Nereis egg. The scale at the upper margin of Fig. 6 indicates the thickness of protoplasm in microns which has an absorbing power equivalent to the thickness of air measured along the lower margin, on the assumption that the cell is 1·2 cm. from the bulb, and the maximum depth of penetration is 0·0065 cm. If our estimations are approximate, and no more is claimed for them, it would appear that after passing half-way to the centre of the egg the strength of the beam of α-rays falls off rapidly and becomes zero at the centre.

Absorption of light. The limitation of the effects of light to one side of the Nereis egg was to be expected so far as the rays of the Schumann region are concerned, for these wave lengths are transmitted through only the most transparent substances. Bovie(8) has given reason to believe that they are completely absorbed by only 10 microns of egg albumen, and that they fail to penetrate into the nucleus of several forms of protozoa. If the rays are absorbed to an equal degree by the substance of the Nereis egg, which is about 130 microns in diameter, their effect would certainly be limited to the side of incidence, just as the effect on the membrane indicates.

The wave lengths of ultra-violet light from the carbon arc which we have found to be effective (between 3100 and 2200 Å.U.) are undoubtedly much more penetrating than those of the Schumann region of the spectrum. Photomicrographs made with light of 2800 Å.U. wave length indicate that the cytoplasm of Paramecium is quite transparent to these rays, but that they are strongly absorbed by the nucleus. Since the absorption of light depends on molecular structure, and this must differ in the different parts of the cell and in cells of different kinds, it is not possible to infer from these observations where the light from the carbon arc will be absorbed by the Nereis egg, and consequently we have re-

[1] It will be noted that the ratio reaches a constant level at about 1·35 at distances beyond 5 cm. This is due to the error in estimating α-rays effects already referred to, and does not indicate a penetration of α-rays beyond this distance. The word strength, as used here, should be taken to mean the energy of the α-ray measured by the fraction absorbed in the layer of protoplasm responsible for membrane-formation. Since the depth of this layer is unknown at present the connotation must necessarily be rather loose.

sorted to direct observation. A photomicrograph of a Nereis egg taken with light of 2800 Å.U. is shown in Fig. 2 *F*. It may be seen that very little light of this wave length penetrates through the thicker, central portion of the egg, even though the egg was somewhat flattened by the cover glass. The light used in making this photograph is in wave length about midway in the region of the spectrum from the carbon arc which we know to be effective. Its absorption by the Nereis egg can be correlated consequently with the fact that the light of the carbon arc affects the membrane of the egg only on the side of incidence.

In the preceding pages we suggest that a fundamental similarity may exist in the mechanisms underlying the action of different kinds of radiation upon living matter, and have advanced in support of this view the fact that α-, β- and γ-rays and ultra-violet light produce qualitatively similar effects upon the process of membrane formation and jelly secretion in the egg of Nereis. The difference which exists between the action of ultra-violet light and of α-rays on the one hand and the action of the penetrating β- and γ-rays on the other, may be explained by the limited penetration of the former into the egg.

The absorption of light by the egg also explains why only the shorter wave lengths of the spectrum are effective. The eggs are quite transparent to light from the visible region, as may be seen from the photomicrograph in Fig. 2 *E*, which was made with light from an incandescent lamp. The light from the carbon arc filtered through glass and from the sun is absorbed to such a slight extent that an insufficient quantity of energy is made available to produce any detectable effect. It is true that β- and γ-rays of radium are probably quite as penetrating as visible light, but the energy available in such radiation is so great that the absorption of a very small portion of it will suffice to bring about a marked physiological effect. There is nothing to contradict the view that visible light would also produce an effect upon the Nereis egg could it be applied with such an intensity that a sufficient quantity of energy would be absorbed by the portion of the protoplasm involved in the reaction in question.

Other effects of α- and β-rays. We wish now to consider the effects of α- and β-rays and ultra-violet light on other processes in the egg and to inquire whether differences in their effects can be explained in a similar manner by differences in the penetrating power of these rays.

Artificial parthenogenesis. When unfertilised Nereis eggs are exposed for appropriate intervals to intense α-radiation by placing them close to the bulb of emanation, a large proportion of them are caused to secrete

their jelly and produce fertilisation membranes which resemble in every particular those formed on eggs which have been fertilised with sperm after exposure to α-rays. If the duration of exposure has been properly chosen many of these eggs proceed to cleave into several irregular blastomeres. The cleavage forms produced in this way are illustrated in Fig. 7 *A* and *B*. They resemble those resulting from severe β-radiation

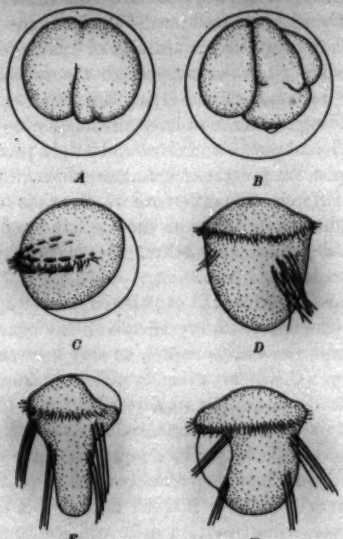

Fig. 7. Based on free-hand sketches of living eggs. *A* and *B* are cleavage forms produced by eggs activated with α-rays.

C is embryo two days after fertilisation; radiated in unfertile condition with α-rays.

D, *E* and *F* are embryos four days after fertilisation radiated in unfertile condition with α-rays.

followed by fertilisation. On exposing eggs to 16·9 millicuries of radium emanation at a distance of 0·55 cm. it was found that optimal exposure was about five minutes. Eggs exposed for much shorter periods fail to be activated. Eggs exposed much longer are activated, but fail for the most part to proceed to cleavage and commence to cytolise within 24 hours. It has been observed that if the radiation is very intense cytolysis

will set in almost at once and before any membrane or jelly formation has occurred.

Loeb(9) has shown that ultra-violet light of wave length shorter than 2607 Å.U. will cause the initiation of development in the unfertile eggs of Arbacia and Chaetopterus. We have had no difficulty in confirming these observations, using the egg of Nereis and ultra-violet radiations from the Schumann region. Four exposures of 15 seconds each at intervals of 2 minutes sufficed to cause every egg to secrete its jelly and form a perivitelline space greatly enlarged on one side. Bovie(5) has shown that various protozoans are very readily cytolised by these radiations.

All our attempts to duplicate the activating effects of α-rays and ultra-violet light by the use of prolonged and intense exposures to β-rays have been without success. After an extensive study of the effects of β- and γ-rays on the eggs of Nereis and Arbacia, Packard(4) concludes that there is no evidence for parthenogenetic development being produced by these rays. Richards(10) also failed in an attempt to activate the eggs of Nereis, Arbacia, and Asterias by the use of X-rays. Bohn(11) claims to have observed 2 to 4 p.c. of unfertile eggs of Strongylocentrotus develop into irregular embryos after exposure to a tube of radium. It is unlikely that the tube containing the radium allowed α-rays to escape so that this effect might be attributed to the action of β- or γ-rays. In view of the small number of eggs which developed in these experiments the possibility of contamination by sperm, the absence of information concerning the control experiments, the fact that a small percentage of certain species of echinoderm eggs tend to develop without fertilisation, and the failure of Packard and ourselves to confirm the observation, great weight cannot be put on this result.

It appears that α-rays and ultra-violet light are effective in producing artificial parthenogenesis and that β-rays, γ-rays and X-rays fail to initiate development in unfertilised eggs. The significance of this difference in these various rays lies in the fact that the α-rays and ultra-violet light are both cytolytic agents which are readily absorbed and exert their effects largely in the superficial layers of protoplasm without causing severe damage to the central parts of the cell. They consequently are able to produce the cytolytic cortical changes which are common to all parthenogenetic agents(12) without injuring too greatly the internal conditions which are necessary for the progression of cleavage and development. β-, γ- and X-rays on the other hand are very penetrating radiations. In passing through the cell they lose very little energy and

affect all parts of the protoplasm almost equally. It is consequently impossible to concentrate their effects on the cortical layers of the cell, as is necessary for the production of artificial parthenogenesis, without at the same time damaging the interior of the cell so greatly that development cannot proceed. In this way it is possible to correlate the activating influence of the α-rays and ultra-violet light with the penetrating powers of these radiations and the apparent specificity of their action becomes merely an expression of the ease with which they are absorbed by protoplasm.

Effects on development. Exposure to severe radiation prevents the eggs of Nereis from proceeding with development, upon fertilisation, beyond a few irregular cleavages and results in the early death of the embryo. We have compared the number of embryos which succeed in developing into ciliated larvae after various lengths of exposure to the different rays from the same bulb of emanation. When this is done it is found that exposure to the entire radiation is very little more effective than exposure to the β- and γ-rays alone after the α-rays have been removed by a thin aluminium filter. In Fig. 8 graphs *B* and *C* show the percentage of embryos which fail to develop cilia in the case of two different experiments. To cause a certain number of embryos to fail to reach this standard of development it is necessary to expose the eggs in the case of the use of β- and γ-rays alone only two or three times as long as when the α-rays are also included in the radiation. Graph *A* shows the effect of the periods of radiation on the thickness of the perivitelline space formed by the eggs used in the experiment upon which graph *B* is based. It shows that the process leading to abnormal membrane formation proceeds about twenty-five times as quickly when α-rays are included in the radiation. The great difference in the velocity of this process and that leading to the failure of the embryo to develop cilia finds a ready explanation from a consideration of the limited penetrating power of the α-rays and the location in the egg of the processes leading to the changes recorded in the two cases.

An examination of the embryos developing from eggs which have been exposed to α-rays shows that the abnormality in the development is limited chiefly to one side, presumably that of incidence of the radiation. Fig. 7 *C* illustrates the appearance of a Nereis embryo exposed to α-rays and allowed to develop for 48 hours. The normal embryo of this stage is completely surrounded by a band of cilia under which occurs a ring of pigment. In the egg illustrated the cilia and pigment have developed normally on one side, but on the opposite side a huge blister

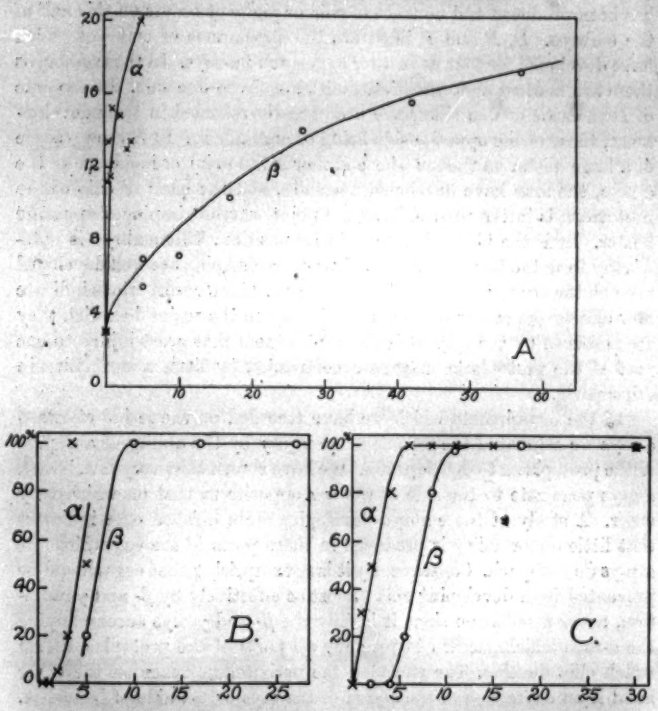

Fig. 8. The relative effect of α- and β-rays on the process of membrane formation and on the development of embryos. α, curves of α-ray effects; β, curves of β-ray effects.

A. Comparison of the thickness of membranes produced by α- and β-radiations from 5·1 millicuries of radium emanation at a distance of 1·12 cm. and of different duration. Thickness of membrane measured in microns along the ordinate; time of radiation measured in minutes along the abscissa. Measurements made 70 minutes after fertilisation.

B. Comparison of number of embryos developing cilia after exposure to α- and β-radiations under same conditions as in *A*. Per cent. failing to develop measured along ordinate, time of radiation measured in minutes along abscissa. Examined 30 to 36 hours after fertilisation.

C. Like *B* except that strength of emanation was 10·5 millicuries. Examined 18–24 hours after fertilisation.

has been produced and no trace of cilia or pigment occurs on this half of the embryo. *D*, *E* and *F* illustrate the appearance of embryos which have developed for four days after exposure to α-rays. In these embryos there are marked abnormalities, limited again to one side. In the case of *D* the setæ of one side have been greatly retarded in their development, those of the opposite side being of normal size. In *E* the presence of a large blister on the anterior end marks the point of incidence of the α-rays, the setæ have developed normally, and the band of cilia of the prototroch is interrupted only at the point where it impinges upon the blister. In *F* the blister is lateral in its position. This embryo is noteworthy from the fact that setæ and prototrochal cilia are well developed even on the very edge of the blistered region. These observations indicate how closely the effects of α-rays are limited to the region in which they are absorbed by the egg. It is clear from them that great injury to one part of the protoplasm may be accompanied by little abnormality in other parts.

In the experiments which we have recorded on the lethal effects of α-rays we estimated the extent of the injury by the ability of any part of the protoplasm to develop cilia. We have shown that very few, if any, α-rays penetrate to the side of the egg opposite to that on which they enter. A study of the embryo developing from α-rayed eggs indicates that little abnormality is produced in those parts of the egg which the α-rays do not reach. Consequently it is not surprising that eggs should be prevented from developing cilia almost as effectively by β- and γ-radiation, as by α-radiation since it is only the β- and γ-rays accompanying the α-rays which succeed in reaching all parts of the protoplasm from which cilia develop. The effect on the perivitelline space on the other hand is an expression of the action of the rays on the side of incidence. The cortical tissues are subject to the full intensity of the α-rays, undiminished by absorption by other parts of the protoplasm. Consequently their reaction gives a truer measure of the relative physiological effects of the incident beams of α- and β-rays.

The difference in the velocities of the reactions leading to abnormal membrane formation and to the failure to develop cilia under the influence of α-, β- and γ-rays and of the β-, γ-rays alone is an expression, not of any specific difference in the ability of these rays to produce reactions, but of the position within the cell of the protoplasm in which the reactions occur, and the ability of rays of the different types to reach these regions.

Conclusions.

1. α-rays and ultra-violet light resemble β- and γ-rays in altering the process of membrane formation and jelly secretion in Nereis eggs, but the process is unaffected by wave lengths of light shorter than 3000 Å.U. The changes which they produce are limited to one side of the egg.

2. Radiation alters the process of jelly secretion and membrane formation by causing a certain quantity of the jelly substance to be retained within the perivitelline space of the egg. The retention of the jelly substance is due chiefly to an alteration in its physical properties. There is no reason to believe that a change in the permeability of the membrane of the egg is an important factor in causing this retention.

3. The differences in the effects of the radium rays and ultra-violet light on membrane formation, on the induction of artificial parthenogenesis, and on the prevention of development are due to differences in the penetrating power of these radiations, and the failure of visible light to affect the egg is attributable to the limited absorption of those wave lengths by protoplasm. Since the change produced in the egg is simple in nature it is justifiable to suspect that some common mechanism underlies the effects produced by these different radiations.

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THE COAGULATION OF THE BLOOD. Part I. The Role of Calcium.

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In all theories of coagulation the presence of calcium salts has been recognised as an essential factor, and it is generally held that the activity of the calcium depends on its presence in the ionised form, though the method of its action is at present not clearly defined. In this paper an account is given of an investigation of the state in which calcium is present in shed blood.

Method of determining the calcium content of blood or serum. The most general method is by incineration of the blood and gravimetric estimation of the lime; this method requires large quantities of material to obtain accurate results, and is unsuitable for repeated examinations in pathological cases. The following method was devised in order to combine rapidity of examination with a simple technique, using 0·5–1 c.c. of blood or serum. The principle of the method is the recalcification of oxalate blood by the addition of the material to be tested, and comparison of the action of the latter with that of calcium chloride solutions of known strength. A series of decreasing dilutions of ammonium oxalate were made up in 0·8 p.c. NaCl solution, commencing at 1/600, and a similar series containing anhydrous calcium chloride, commencing at 1/5000. Normal blood was obtained from the author's finger, without undue pressure. The blood was drawn into pipettes made of thermometer tubing and divided into 30 divisions, 15 of which were equal to 0·08 c.c., or each division to 5·33 c.mm. The experiments were carried out in tubes about 5 mm. in diameter. The extent of coagulation was marked as 0, no clot; *a*, slight clot; *b*, marked clot; *c*, nearly complete clot; *d*, complete clot. The amount of oxalate or calcium given is the number of milligrams per 100 parts of blood which must be added to produce the described effect. The estimation of the calcium content of an unknown fluid therefore consists of three parts.

A. To a series of tubes each containing 10 vols. of normal blood, 4 vols. of ammonium oxalate solution of decreasing concentration were added, and the minimal amount of oxalate noted, which prevented clotting for a given time. This was found to be 57·1 mg. p.c.:

Exp. 1 *a*. Ammon. ox. mg. p.c.	57·1	50·0	44·4
37° C. mins. 4	0	0	0
6	0	*b*	*b*
8	0	*c*	*c*
10	0	*d*	*d*

B. To a second series of tubes each containing 10 vols. of normal blood and the minimal amount of ammonium oxalate which prevents clotting (57·1 mg. p.c.), 2 vols. of calcium solutions of decreasing concentration were added. An additional control tube contained 10 vols. of normal blood and 6 vols. of 0·8 p.c. NaCl solution. The tube in which coagulation was complete at the same time as in the control, showed the minimal amount of calcium required for complete coagulation in the given time, as in the following example.

Exp. 1 *b*. Ammon. ox. = 57·1 mg. p.c.

Ca mg. p.c.	0·66	0·61	0·56	Control
37° C. mins. 4	*a*	0	0	*a*
6	*c*	0	0	*c*
8	*d*	0	0	*d*

The minimal amount of calcium was here 0·66 mg. p.c. This amount is almost constant; over a period of three months it varied in the author's case between limits of 0·61–0·66 mg. p.c.

C. To a third series of tubes blood and oxalate were added as in A, and to this 2 vols. of the unknown blood or serum were added after proper dilution, instead of the calcium solution in 1 *b*; as the volumes of the calcium solution and the diluted unknown fluid are the same, their calcium contents must also be identical at that dilution where complete clotting is just produced in the same time in each. In estimating the calcium content of blood or serum where free thrombin may be present, it is necessary to heat the fluid at 55° C. for an hour previous to the experiment.

The determination of complete coagulation by observing the time when there is no free fluid present on inverting the tube is inaccurate, since there may be adhesion of the fluid to the vessel wall without true clotting. It has been found more satisfactory to make the final reading by filling the tubes with water, when the amount of true clot may be seen. When coagulation is complete there will be practically no discolouration of the added water, even if the tube is gently shaken.

The method described is not claimed to be of the same degree of accuracy as the gravimetric methods, as its aim is to enable the investigator to deal rapidly with small amounts of blood. The following table shows the error per 100 parts of solution in estimating the calcium content of solutions of known strength:

Known strength mg. p.c.	Average error p.c. in estimation
9·091	0·199
7·273	0·273
6·061	0·172
5·195	0·148

This gives a total error of 0·198 mg. in 6·9 mg. of calcium, or an average error of 2·87 mg. per 100 mg. of calcium.

Calcium content of serum. Using the method described, the calcium was estimated in the sera of a series of healthy adults. 0·5 c.c. of blood was used in each case, and the amounts of calcium found were

10·72, 10·76, 10·58, 10·76, 10·78, 10·21, 10·66, 10·66.

These figures show that the serum-calcium is fairly constant in different individuals, and is in agreement with the work of Howland and Cramer(1). In a series of seven adults, these workers found a variation between 9·3 and 9·9 mg. Ca p.c.; using 2 c.c. of serum, the calcium was estimated by an incineration and subsequent titration with permanganate. Howland and Marriott(2) using a colorimetric method, found a variation between 11·3 and 9·2 mg. p.c. The serum-calcium seems also to be relatively constant in the same individual over a considerable period; in the author's case in three months the variation was between 10·55 and 10·77 mg. p.c.

Taking the calcium content from these figures as 10·8 mg. and the minimal amount of calcium necessary to cause clotting as 0·66 mg., only 6·1 p.c. per 1/17th of the total available calcium percentage of the blood is necessary in order to restore coagulation after complete oxalation. It is clear therefore that the recalcification process is not a quantitative replacement of the calcium precipitated by the oxalate, but a more complex process which will be discussed later.

Calcium content of whole blood before coagulation. If whole blood is heated to 55° C. for an hour, coagulation is prevented probably by precipitation of the fibrinogen, and will not proceed on the addition of calcium salts. A series of blood specimens was taken and the first one placed at once in the 55° C. incubator; the remaining specimens were incubated for varying periods at 23° C. and then subjected to 55° C. The calcium content of each was then estimated by the method described. Table I and Fig. 1 show the results obtained from three experiments, with three estimations before clotting in addition.

These results show that as coagulation proceeds, the content of ionised calcium in the fluid progressively increases until it reaches the value found above for serum, when it remains constant. The conclusion seems inevitable that part of the blood calcium is in combined form, that the combination is broken down during clotting with the liberation of ionic calcium, and that calcium does not enter into the chemical composition of the clot.

TABLE I.

Time at 23° C. mins.	Clot	mg. Ca p.c. I	II	III	IV
0	0	6·69	6·61	6·61	6·61
—	—	—	—	—	7·21
—	—	—	—	—	6·71
1	0	—	6·61	—	—
2	0	7·46	—	7·05	—
3	0	—	7·27	—	—
4	*a*	9·70	—	8·14	—
5	*b*	—	9·22	—	—
6	*c*	10·77	—	8·81	—
7	*c*	—	9·92	—	—
8	*d*	10·77	—	10·58	—
9	*d*	—	10·77	—	—
12	—	10·77	10·77	10·58	—

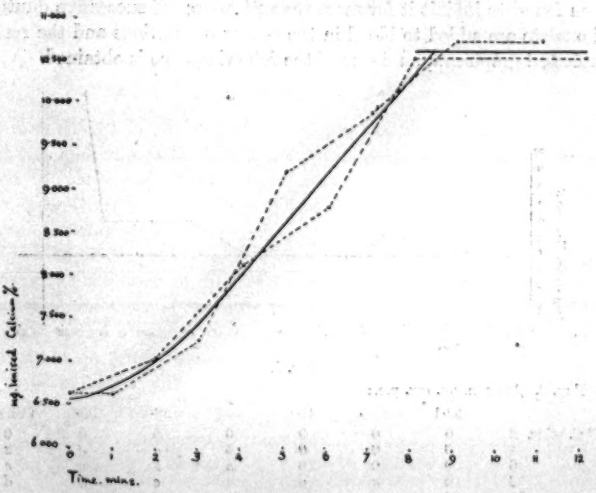

Fig. 1.

The data given above show an average ionic calcium value of 6·74 mg. p.c. before coagulation and 10·74 mg. p.c. after coagulation, so that immediately after blood is shed, 4 mg. or 37·4 p.c. of the available calcium in normal blood are combined and in a non-ionised state. Similar estimations in a series of normal adults gave comparable results.

TABLE II.

Obs.	Ca value before clotting	Additional Ca value after clotting
1	6·74	4·00
2	6·69	3·52
3	6·46	4·32
4	6·82	3·84
5	6·82	3·84

Effects of varying the oxalate concentration. When a soluble oxalate is added to a solution of a calcium salt, a precipitate of calcium oxalate is at once thrown down, and it is reasonable to suppose that the same reaction takes place in the case of the ionised calcium of the blood. But in regard to the combined calcium the reaction is more complex. The amount of oxalate used in the method described is not an amount which merely alters the coagulation time from normal, but which has been found to inhibit it for more than 24 hours. If successive dilutions of oxalate are added to blood in the proper proportions and the results expressed graphically, a figure of the following type is obtained.

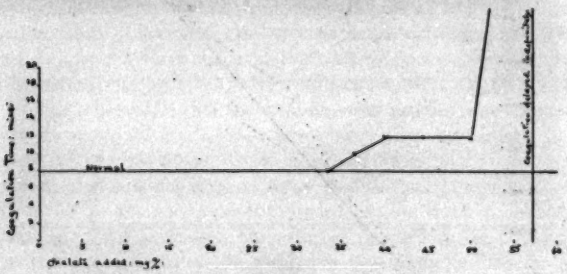

Fig. 2.

Exp. 2. Ammon. ox. mg. p.c.:

	57·1	50·0	44·4	40·0	36·4	33·3	Control
37° C. mins. 2	0	0	0	0	0	0	0
4	0	0	0	0	*a*	*a*	*a*
6	0	0	0	*a*	*b*	*c*	*c*
8	0	*a*	*a*	*a*	*c*	*d*	*d*
10	0	*c*	*c*	*c*	*d*	—	—
12	0	*d*	*d*	*d*	—	—	—

In this experiment two distinct changes occur in the coagulability of the blood; first a slight prolongation of the clotting time and second a practically complete inhibition of coagulation. Taking the total calcium content of the blood under consideration as 10·74 mg. Ca p.c. (Table I), and assuming that one molecule of oxalate combines with

one atom of calcium, 33·29 mg. of oxalate would combine with the total calcium of the blood. But since clotting was not prevented by the addition of 33·3 mg. of oxalate, it follows that the precipitation of the ionised fraction of the total calcium has no effect on the clotting process. The part played by calcium in coagulation must therefore be confined to the combined fraction.

It has been shown (Table II) that the ionised calcium content is 6·74 mg. p.c. If one molecule of oxalate combines with one atom of calcium, 20·89 mg. of oxalate will combine with the ionised calcium. Subtraction of this amount from the oxalate added in Exp. 2 shows the effect of the oxalate on the combined calcium and the clotting time.

TABLE III.

Ammon. ox. less equiv. of ionic Ca mg.	Clotting time mins.	Molecular ratio combined Ca (4 mg.) to oxalate
12·41	8	1 : 1·0
15·51	10	1 : 1·25
19·11	12	1 : 1·5
23·51	12	1 : 1·9
29·11	12	1 : 2·4
36·21	infinity	1 : 3·0

The first change in the coagulation time therefore occurs when more than one molecule of oxalate per atom of combined calcium is introduced into the system, but from that point the coagulation time remains more or less constant until three molecules are present, when it becomes delayed indefinitely.

Effect of varying the time of exposure to oxalate. In Exp. 1 the time between the oxalation of the blood and the addition of the calcium was as short as possible. If exposure to oxalate is prolonged, Exp. 3 shows that increasing amounts of calcium must be added to bring about coagulation, in proportion as the period of exposure is increased. Here the blood is mixed with a constant concentration of oxalate, and then incubated for varying periods.

Exp. 3. Oxalate concentration = 57·1 mg. p.c.

Time at 37° C. mins.	mg. Ca added for coagulation
0	0·66
5	0·81
10	0·91
15	0·97
30	1·12
45	1·45
60	1·82

The average value of three estimations after 60 mins. was 2·025 mg. The explanation of these results is probably as follows. It has been

shown that 57·1 mg. of oxalate are required to prevent clotting, and that of this 20·89 mg. combine with the ionised calcium. Therefore the remaining 36·21 mg. must unite with the combined calcium; but since the blood has been shown to contain 4 mg. of combined calcium, within the limits of experimental error three molecules of oxalate have united with one atom of combined calcium. On this basis the oxalated combined calcium complex can be represented by the formula $R.Ca.Ox_3$ where R is an unknown organic substance. This holds good only after oxalation which delays coagulation indefinitely. When the exposure to oxalate is minimal, as in Exp. 2, coagulation occurs at a calcium-oxalate ratio of 1 : 1 for the combined calcium, the formula therefore being R.Ca.Ox.

A comparison of the results of calculation from these formulæ gives close agreement with the experimental results. In Exp. 1 it has been shown that clotting can still occur at an oxalate concentration of 50 mg.; since the ionised calcium has been precipitated, the combined calcium is represented by 50 − 20·89 mg. = 29·11 mg. of oxalate. But 36·21 mg. of oxalate are equivalent to 4 mg. of combined calcium, so that 29·11 are equivalent to 3·22 mg.; therefore the amount of combined calcium which is still active at an oxalate concentration of 50 mg. p.c. is 4 − 3·22 = 0·78 mg. The calcium found by experiment was 0·66 mg. If the formula $R.Ca.Ox_3$ is the same when exposure to oxalate is minimal or prolonged, the difference in the amount of calcium which must be added to cause clotting in the two cases, must lie in a change of physical state in the oxalated complex. When exposure is minimal, the oxalate molecules are loosely attached to the complex: it has been shown (Table III) that clotting can occur when the combined calcium has one molecule of oxalate attached, so that sufficient calcium must be added to remove two of the oxalate groups; the complex can then enter into the clotting process, and in so doing liberates ionised calcium which in turn combines with other oxalate groups; this reaction is therefore progressive and requires only 0·66 mg. of calcium to start it. When exposure to oxalate is prolonged, the oxalate groups are more firmly combined, so that one of them must be completely removed from the complex; therefore the amount of calcium added must be equal to one-third of the total combined calcium, *i.e.* 1·33 mg. Such addition produces the loose form of combination mentioned above; so that a further 0·66 mg. of calcium must be added to cause clotting; the total amount would therefore be 1·99 mg.; experimentally the amount was found to be 2·03 mg. The correspondence of calculation and experi-

ment confirm the view that calcium exists partly in combination with an organic radicle, which has the power of adsorbing oxalate in excess of the equivalent amount of calcium attached to it.

In Exp. 3 the maximum exposure to oxalate was one hour; in Exp. 4 a longer time was allowed and the oxalate concentration increased in order to see if any further saturation of the complex took place.

Exp. 4. Time at 37° C. constant = 24 hours.

Excess oxalate	Ca equiv.	Ca used in exp.
9·57	3·07	3·23
15·63	5·04	5·82
22·90	7·40	8·31
31·79	10·26	12·12

The first column shows the oxalate added in excess of 57·1 mg. p.c. Since the values of the calcium equivalent of the excess oxalate and the calcium used in the experiment agree, it may be said that the saturation of the calcium complex is complete after one hour's exposure to an oxalate concentration of 57·1 mg. p.c.

The relation between the calcium and the organic radical may be either that of an adsorption compound or of a definite chemical combination. As regards normal coagulation the former seems the more probable, for evidence has been put forward which suggests that the organic complex is very readily broken down during the coagulation process, and that free ionised calcium is liberated. There is also some evidence that change occurs in the physical state of the complex under certain conditions. The function of the calcium is probably that of an inorganic catalyst accelerating a physico-chemical reaction. The linkage between the calcium and its organic component is probably an unstable one in normal untreated blood, and it is possible that changes in surface tension, temperature and other similar factors when blood is shed or vascular injury occurs, may be sufficient to bring about the union of the calcium complex with another proteid material, such as thrombogen, thus setting the train of coagulative processes in motion with the ultimate precipitation of fibrin. A similar theory has already been put forward by Bloch(3).

Effect of dilute acid and alkali. Dilute acid and alkali have the power of splitting off the calcium from the complex. Normal blood was heated to 55° C. to prevent clotting and the calcium content was estimated. It was then exposed to the action of dilute caustic soda for varying times; the best results are obtained with *N*/10 soda. The blood was then neutralised with an equal volume of *N*/10 HCl, and heated to 55° C. for an hour. The ionised calcium was estimated again.

Exp. 5. Blood = 60 vols. $N/10$ NaOH = 10 vols.
$N/10$ HCl = 10 „

Exposure to NaOH hours	Ionic Ca mg. p.c. before NaOH	Ionic Ca mg. p.c. after NaOH
2	6·61	10·40
3	6·80	10·40
6	6·61	11·18
24	6·80	10·10

These results give an average value of 6·7 mg. p.c. Ca before exposure to soda and 10·5 mg. after, showing a close similarity to the values obtained in Exp. 2.

In certain pathological conditions the treatment of the serum by this method also causes an increase in the ionic calcium content which should not normally occur, as in the following instance. The untreated serum was found to contain 6·01 mg. p.c. ionised calcium. It was treated with one-sixth its volume of $N/10$ NaOH and incubated over night at 37° C. After neutralisation with an equal volume $N/10$ HCl, the calcium was again estimated and found to be 9·45 mg. Ca. If in accordance with what has been said, 4 mg. of calcium had been set free during clotting, 7·44 mg. out of a total of 9·45 mg. of the blood calcium was in the combined form and the ionised calcium was proportionately decreased. In this case injections of ionised calcium salts were given, causing an increase in the ionised calcium of the blood and an improvement in the patient's condition. In Table IV a series of such estimations in pathological sera is given.

TABLE IV.

Ionic Ca before treatment mg. p.c.	Additional Ca in mg. p.c. after treatment with $N/10$ HCl	Additional Ca in mg. p.c. after treatment with $N/10$ NaOH
6·01	—	3·44
6·01	—	3·00
7·35	1·32	1·68
6·98	—	1·29
6·01	2·66	3·01
6·62	1·65	2·05

The figures given by the action of the acid, though close to those given by the alkali, are slightly lower. For routine purposes the alkali was used and its action continued over night. In pathological conditions it is probable that the calcium is not combined with the same organic substance as in the normal complex.

Effect of alkali on oxalate blood. It has been shown that dilute alkali or acid is able to split off the combined calcium from the complex completely in two hours. Normally clotting occurs in a few minutes and is accelerated by these reagents. It is possible that the early stages

of their action constitute a loosening of the calcium linkage so that union of the organic radicle with the thrombogen factor occurs more readily. This accelerator action is shown in the case of oxalate blood after addition of decreasing amounts of ionic calcium.

Exp. 6.	A. No NaOH added. Oxal. conc. 57·1 mg. p.c.				B. 2 vols. *N*/10 NaOH added. Oxal. conc. 57·1 mg. p.c.			
mg. Ca added p.c.	0·73	0·66	0·61	0·56	0·73	0·66	0·61	0·56
37° C. mins. 2	0	0	0	0	0	0	0	0
4	0	0	0	0	*c*	*b*	0	0
6	*b*	*b*	*a*	*a*	*d*	*c*	0	0
8	*d*	*d*	*a*	*a*	—	*d*	*a*	0
10	—	—	*b*	*a*	—	—	*c*	0
12	—	—	*b*	*a*	—	—	*d*	0
15	—	—	*b*	*a*	—	—	—	*a*
20	—	—	*b*	*a*	—	—	—	*c*
30	—	—	*c*	*b*	—	—	—	—

Clotting is accelerated in all cases in B where soda has been added. This experiment was carried out under conditions of minimal exposure to the action of the soda and oxalate.

The action of dilute alkali or acid does not change the properties of the organic radicle of the complex fundamentally; this is shown by the fact that after prolonged treatment with these reagents clotting will occur normally on the addition of sufficient calcium. Exp. 7 will serve as an example.

Exp. 7. Amm. ox. 57·1 mg. p.c.
Blood. 10 vols.
N/10 NaOH. 2 „
} 37° C. for 24 hours.

Ca added mg. p.c.	7·26	5·00	3·63	Control (no NaOH) 2·41
37° C. mins. 4	0	0	0	0
6	*c*	*b*	0	*b*
8	*d*	*c*	0	*c*
10	*d*	*d*	0	*d*
20	—	—	*a*	—

This experiment shows that 5 mg. of calcium must be added to cause clotting; the same result was obtained using *N*/10 HCl instead of soda. Under the conditions of the experiment the calcium should have been completely split off from the complex, and since the blood is fully oxalated the separated calcium must also be oxalated and not available for clotting. Assuming that the calcium of the complex bears one oxalate molecule and the organic radicle two, it will be necessary to replace the whole of the combined calcium (4 mg.), and since the newly formed complex now bears two oxalate groups, clotting will occur on adding a further 0·66 mg. of calcium, in all 4·66 mg. This is supported by the experiment.

If the acid or alkali is neutralised before the calcium is added, a different result is obtained, shown in Exp. 8. The same process was followed as above except that an equal volume of $N/10$ HCl was added immediately before recalcification.

Exp. 8.			Control oxalate only
Ca added mg. p.c.	2·41	1·81	2·41
37° C. mins. 4	0	0	0
6	*b*	0	*b*
8	*c*	*a*	*c*
10	*d*	*a*	*d*
15	—	*b*	—

A similar result is obtained with dilute acid and neutralisation by soda. Since it has been shown that complete separation of the calcium from the complex occurs under the conditions of the experiment, neutralisation of the blood must allow the calcium to reunite, for the same amount of calcium is required as in the control where separation has not been able to occur. The same amount of calcium is necessary to cause clotting as in the case of prolonged exposure to oxalate (Exp. 3): therefore the separated calcium reunites to the complex, and with it the third oxalate group which was shown to be split off in Exp. 7. This supports the suggestion that the complex has two components, of which the organic part adsorbs two molecules of oxalate and the calcium, one. Further evidence is obtained from Exp. 2. Here complete inhibition of coagulation occurs suddenly when three molecules of oxalate have been added for each atom of combined calcium. This indicates that the calcium atom of the complex enters last into combination with the oxalate: *i.e.* that the organic part of the complex will take up two molecules of oxalate before the calcium is affected, and that it is the final combination of the calcium which causes the complete inhibition of coagulation.

Action of the fluorides. Potassium fluoride was used in these experiments on account of its greater solubility. If however an attempt is made to dissolve a relatively large amount of the salt in a small quantity of normal saline, a precipitate of sodium fluoride is formed and the fluoride content of the solution becomes inaccurate. The solutions were made up in distilled water, which introduces a minor inaccuracy by diluting the salts of the blood. Further on adding the fluoride to the blood, the magnesium will probably be precipitated as well as the calcium since their solubilities are approximately of the same degree, thus causing another error. Finally the fluorides have a tendency to attack the glass vessels in which they are contained. Realising these sources of error,

by following the same technique which has been described for the oxalates, the following figures were found:

Minimum amount of KF which inhibits clotting for 10 mins. ... ... = 571 mg. p.c.

Minimum amount of Ca necessary to cause clotting under these conditions ... ... = 0·66 mg. p.c.

Assuming that the equation

$$2KF + CaX = CaF_2 + K_2X,$$

where X is an ionised acid radicle, is followed, the amount of fluoride necessary to combine with the ionised calcium is found to be 19·4 mg. p.c. leaving 551·6 mg. to combine with the combined calcium. As it has been shown that there are 4 mg. of combined calcium in the blood used, the molecular ratio of calcium to fluoride is as 1 to 95 instead of the normal 1 to 2. On the assumption that the same change occurs on prolonged exposure to fluoride as in the case of the oxalates, it can be calculated that the amount of added calcium necessary to turn out the fluoride would be 191 mg. p.c. or 523 mg. of calcium chloride. It may be for this reason that the theory that the fluorides adsorb the clotting ferments, has arisen.

Action of the citrates. It is stated that the citrates prevent clotting by forming a double salt with the blood calcium; this salt is soluble but ionises in such a way that free calcium ions are not present in the solution. In this work ammonium citrate was used and the same technique followed as before. The amount of citrate necessary to prevent clotting was found to be 94 mg. p.c.; of this the ionised calcium would require 23 mg., leaving 71 mg. to combine with the combined calcium. This gives a ratio of three atoms of combined calcium to ten of citrate; the normal ratio chemically is three of calcium to two of citrate. The amount of calcium added to cause clotting under these conditions and also in the case of the fluorides was 0·66 mg. p.c. This suggests that this calcium value is an attribute of the clotting complex and independent of the inhibitor used.

Calcium citrate is relatively insoluble, but using a saturated solution of the salt, normal blood was not prevented from clotting by the addition of this citrate solution. When oxalate blood was used (57·1 mg. p.c.) clotting was readily caused by the addition of the calcium citrate solution, and at an oxalate concentration of 66·7 mg. p.c. complete coagulation was just produced in ten minutes by the use of the same reagent. This indicates that calcium citrate ionises to an appreciable extent, and

that the effect of the calcium ion predominates. With the object of obtaining a solution of the double citrate compound with calcium, millenormal solutions of the two reagents were mixed in the proper proportions. After two hours at 37° C. calcium citrate was precipitated from the solution. The action of this solution to oxalate blood was again that of a solution containing free calcium ions.

It is difficult to find definite evidence that a double salt of sodium calcium citrate is formed, or that the citrates exert their anti-coagulant action in this way. It has been shown that the citrate is adsorbed to the molecule of the combined calcium, and that by such combination the function of the clotting complex is suspended. If calcium citrate ionises and if the action of the calcium ion is predominant, the citrated ionised-fraction of the blood calcium should be able to relieve the clotting complex of its adsorbed citrate and clotting should take place. But it must be remembered that the amount of citrate added is large and that there is present a considerable excess of citrate over the total calcium content of the blood. The relation of the ionised calcium and the ionised citrate to the combined calcium and adsorbed citrate is probably a balanced one when the blood is fully citrated, so that even though ionised calcium may still be present it is not sufficient to overcome the action of the adsorbed citrate since there is an excess of citrate in the system, and it is only by increasing the calcium ion concentration by the addition of fresh calcium that the action of the citrate is overcome and coagulation proceeds. This would imply that a substance acts as an anticoagulant in virtue of some structural character of its molecule, for it may be noticed that the carbonates are unable to prevent clotting, even though calcium carbonate is less soluble than the fluoride. Following the present theory, this lack of anticoagulant action is due to the inability of the carbonate group to combine with the clotting complex, and not because the carbonate formed may be the soluble hydrogen carbonate.

Summary.

1. Calcium is present in normal blood in two forms, ionised and combined; the latter is transformed into the ionic state during coagulation.

2. The addition of oxalate, citrate or fluoride to normal blood in amount chemically equivalent to the total calcium of the blood does not inhibit coagulation; this indicates that the presence of ionised calcium is not essential to the clotting process.

3. The anti-coagulants described all inhibit clotting primarily by combining with the calcium-containing complex; the amount of anti-coagulant required to neutralise the action of the complex is in each case in excess of the chemical equivalent of the calcium attached to it.

4. Calcium combined with an organic substance at present not defined is the essential factor in the commencement of blood coagulation; this complex probably corresponds to the thrombokinase of Morawitz.

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THE OXYGEN USE OF MUSCLE AND THE EFFECT OF SYMPATHETIC NERVES ON IT.

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Mansfeld and Lukács have recently given an account of some experiments which, they hold, confirm the theory of de Boer that the sympathetic nervous sytem exercises a tonic action on striated muscles. They estimated the respiratory exchange in the dog, as shown by analyses of the respired air. Curari was given in quantity just sufficient to paralyse the sciatic nerve. They found that section of the sciatic when the sympathetic was intact caused a decrease of respiratory exchange, but that the section when the lumbar sympathetic had been cut, had no effect on respiratory exchange. They concluded that the intact sympathetic increased the oxygen use of the muscles. There is, however, another possible explanation of the result. If, on cutting the sympathetic nerves, there was dilatation of the blood vessels of the limb, there would be transference of blood from the viscera to the limbs, *i.e.* from a region of more active metabolism to one of less active metabolism and a decrease of respiratory exchange would naturally follow.

It is clear that in order to decide whether the sympathetic affects the oxygen use of the muscles, direct determination of the oxygen in the blood flowing to and from the muscles is required. On the data given by Mansfeld and Lukács, the change in the oxygen consumption by the muscles would be completely outside the limits of experimental error. They found that the respiratory exchange of the respired air decreased 10 to 15 p.c. on section of the sciatic with its sympathetic fibres, and attributed the whole of this decrease in oxygen use to a decrease in the oxygen use of the muscles of the limbs.

My experiments were begun at Professor Langley's suggestion from this point of view, and in the course of them I have made observations on the oxygen use of skeletal muscle in other conditions than section of the sympathetic. The experiments were made on cats. The animals were either anæsthetised with urethane (1·5 to 2 grms. per kilo body

weight given subcutaneously) and then if necessary with A.C.E. mixture, or they were anæsthetised with chloroform, decerebrated by Sherrington's method, tracheotomy performed, curari given and the anæsthetics discontinued.

In order to obtain blood coming exclusively, or almost exclusively, from the muscles, the following procedure was adopted:—The cord extending the limb was drawn tight around the ankle so as to exclude the blood coming from the foot. The veins in the popliteal space were exposed, the small saphenous (ext. saph.) vein was tied a little below its junction with the popliteal, a cut made in the vein near the junction, and a clamp placed on the vein above the cut. A woollen thread was passed under the popliteal vein. The venous blood was collected in the manner introduced by Barcroft and Kato (cp. their Fig., *op. cit.* p. 150) by inserting the neck of a graduated pipette through the cut in the small saphenous vein past the valve at its popliteal junction. The blood was diverted from the popliteal into the small saphenous by an assistant pulling up the thread lying under the popliteal as given by Langley and Itagaki. By this method the gastrocnemius is very little exposed, and venous blood is obtained from extensor and flexor muscles of the leg and from the bones but not from the skin. It is possible that some blood may have passed to the popliteal vein from the foot, but I found no colour in the foot (or skin of the leg) on forcibly injecting 2 p.c. methylene blue after death into the lower end of the small saphenous vein, though the muscle-veins of the lower leg were well injected. The same pipette was used throughout; 2 p.c. potassium oxalate was passed through it before the first sample of blood was taken, it was washed after each sample, and again rinsed with potassium oxalate, thus differences in the rate of flow caused by a different diameter of the nozzle of the pipette were avoided. After each sample of blood, care was taken to see that the thread under the popliteal vein caused no obstruction to the blood flow. The rate of flow of each ·1 c.c. was recorded on a kymograph. A few minutes after collecting the venous blood, a sample of arterial blood was taken from the carotid artery by a hypodermic syringe and the oxygen use of the blood determined by Barcroft's differential method. The preliminary operation took 1 to 1½ hours; an interval of about 20 mins. was then allowed before taking the first blood sample. The animal was kept warm throughout.

As the protracted nature of the experiment and the withdrawal of a number of samples of blood might cause a decrease in oxygen use, it was necessary in the first place to determine the course of events

when the nerves were left intact. The results of such experiments are given in Table I.

TABLE I. Cats. Urethane, nerves untouched.

Exp. 1. Wt. 2·6 kgm.

Hrs.	mins.	Blood flow per min. c.c.	O_2 use per min. c.c.
	0	4·95	·119
	30	3·52	·131
1	30	2·57	·123
2	0	1·84	·115
2	30	2·60	·116
3	30	1·67	·113
4	0	1·06	·093

Exp. 2. Wt. 2·7 kgm.

	0	5·60	·168
	30	4·05	·163
1	0	3·46	·168
2	0	2·29	·159
2	30	1·81	·168
3	0	1·46	—
3	30	1·13	·122
4	0	0·53	·061
4	30	0·78	·088

Exp. 3. Wt. 2·5 kgm.

Hrs.	mins.	Blood pr.* in mm.	Temp.	Blood flow per min. c.c.	O_2 use per min. c.c.
	0	151	96·4	—	—
	30	139	96·9	6·00	—
1	0	116	97·0	2·19	·138
1	30	109	98·0	1·75	·139
2	0	110	97·7	—	—
2	30	118	97·0	2·26	·137
3	0	114	96·5	2·09	·140
3	30	128	96·0	1·41	·134
4*	0	—	96·1	1·81	·140
4	30	116	95·8	1·45	·130
5	0	117	95·8	1·22	·130
5	30	94	95·6	1·60	·137
5	50	—	—	1·23	·132
6	10	—	—	1·37	·132

Exp. 4. Wt. 1·9 kgm.

	0	158	—	—	—
	30	115	95·8	7·00	·091
1	0	119	95·7	3·65	·101
1	30	93	95·7	2·29	·105
2	0	81	95·8	2·09	·108
2	30	72	95·9	1·50	·108
3	0	67	95·4	1·29	·109
3	30	58	95·4	·79	·099

* The blood pressure, except the first, was that immediately after the sample of blood was taken. The temperature was taken in the anus.

It will be seen that the rate of blood flow declined fairly steadily, but that the rate of oxygen use did not decline with it. Naturally when the decrease of oxygen supply is sufficiently great a decrease in oxygen use must occur, but the only experiment in which this occurred to any marked extent was Exp. 2. The obvious deduction from the results is that in quiescent muscle, the oxygen use is within wide limits independent of the oxygen supply. Barcroft and Müller found in the sub-maxillary gland that increase of oxygen supply did not cause an increase of oxygen use, and Verzár that decrease of oxygen supply did not cause decrease of oxygen use. The condition in the muscle seems then to be the same as in the submaxillary gland. This conclusion is contrary to that arrived at by Verzár. He found, in the few experiments made on muscle that decrease of oxygen supply (by decreasing the percentage of oxygen in the inspired air) decreased the oxygen use. His results pointed to a close dependence of the oxygen use of the muscle on the

oxygen brought to it. If the rate of blood flow in my experiments had been much greater than in his, the difference in result might be partially reconciled since he did not investigate the effect of increased blood supply. The rate however was not very different.

In Verzár's experiments the rate of blood flow from the gastrocnemius varied from ·6 to 6 c.c. a minute. The gastrocnemius (triceps suræ) forms not quite half the area from which the blood was collected in my experiments, so that if the rate of flow were the same as in his, the flow per min. would have varied from a little less than 1 to nearly 12 c.c. a min. In my experiments the blood flow was well within these limits, but decrease of oxygen supply by reduced blood flow caused no decrease in oxygen use. Further in Verzár's mode of experiment the muscle is necessarily much exposed. In mine, most of the muscles were not exposed at all, and the gastrocnemius but little. It is reasonable then to hold that my experiments represent the normal conditions more nearly than do those of Verzár.

The great variation in the rate of blood flow found in different experiments in cats of the same weight makes estimation of the normal rate in quiescent muscle extremely uncertain. There are a number of obvious factors—unnecessary to recount—which cause a variation in it. It may be doubted whether in waking life the blood flow can ever be as low as that giving only the minimal oxygen requirement of muscle not engaged in replacing lost substance. In experiments, new factors causing either an increase or decrease are introduced. An estimate of the average rate of circulation per gram of soft tissue in the body per minute can be made roughly from the data that the blood is 5 p.c. of the body weight and the circulation time 20 secs. Allowing for the absence of circulation in epidermis and cartilage, and the weight of bone in the body, the average circulation rate in soft tissues could hardly be less than 0·2 c.c. per gram per minute. If this had been the rate in my experiments (reckoning bone as having the sp. gr. of muscle), the rate of blood flow, calculated on the ratio of muscle to body weight given by Langley and Hashimoto (This *Journ.* 52. p. 15, 1918), would have been about 5 c.c. a minute for cats of 2 kgm. and about 8 c.c. a minute for cats of 30 kgms. As will be seen from the tables the rate was generally but not always less than this. In Langley and Itagaki's experiments, in which the blood flow was taken from about the same area as in mine, the lowest rate of flow was 3·93 c.c. a minute.

The effect of section of the sympathetic and of the sciatic nerves was next tried. The sympathetic was dissected from the back so as to avoid exposure of the viscera. The incision was made about 2 cm. from the mid-line at the level of the 6th lumbar vertebra, and extended upwards 6–8 cm. The aponeurosis was cut, and the muscles separated from the transverse process of the 6th lumbar vertebra. The transverse process was cut away. The sympathetic trunk was then isolated from the 5th to the 7th transverse process, care being taken not to puncture the peritoneum. In small cats, the transverse process of the 5th lumbar

vertebra was also removed, a loose ligature was put round the sympathetic between the 5th and 6th lumbar ganglia so that when required it could be gently pulled away from the surrounding tissue and cut. In cutting it during an experiment, the viscera were not disturbed. The place of section was confirmed by post-mortem examination.

TABLE II. Cats. Urethane. Sympathetic and sciatic cut.

Exp. 1. Wt. 2·4 kgm.

Hrs.	mins.	Blood flow per min. c.c.	O_2 use per min. c.c.
0		0·94	·041
10		0·38	·041
20		Both symp. cut	
23		0·44	·046
38		0·46	·047
40		Sciatic cut	
44		0·46	·047
1	10	0·42	·042

Exp. 2. Wt. 2·0 kgm.

0		2·48	·071
10		2·05	·070
30		Symp. cut	
35		1·30	·063
1	0	0·98	·063
1	10	Sciatic cut	
1	15	0·95	·063
1	35	0·54	·072
		Opposite side	
1	55	0·43	·062
2	10	0·55	·070

Exp. 3. Wt. 2·3 kgm.

Hrs.	mins.	Blood flow per min. c.c.	O_2 use per min. c.c.
0		3·00	·061
20		1·86	—
30		1·35	·059
40		Symp. cut	
43		0·60	·060
1	10	0·58	·063
1	40	0·63	·061
1	50	Sciatic cut	
2	5	0·61	·069
2	20	0·42	·063

Exp. 4. Wt. 2·9 kgm.

0		9·14	·103
10		6·32	·098
20		6·46	·106
30		Both symp. cut	
1	3	Sciatic cut	
1	6	4·47	·099
1	19	3·76	·103
1	27	3·86	·097
2	9	3·40	·109
2	17	2·39	·098
2	25	2·93	·107
3	10	2·98	·105
3	19	2·31	·099
3	27	1·98	·109

Exp. 5. Wt. 2·7 kgm. 1st blood sample; blood flow 10 c.c., O_2 use ·155, there was movement during the collection of one, and only one, sample. This caused an increase of blood flow from 5·95 c.c. to 7·90 c.c., and of O_2 use from ·151 c.c. to 242 c.c.

Exp. 6. Wt. 2·5 kgm. 1st blood sample; blood flow 5·33, O_2 use ·130.

Exp. 7. Wt. 2·2 kgm. „ „ „ 3·50, O_2 use ·092.

TABLE III. Decerebrated and lightly curarised.

Exp. 1. Wt. 2·0 kgm.

0	70 c.c. Ringer's fluid injected into jugular vein	
15	1·31	·062
18	Symp. cut	
19	1·14	·063
25	1·13	·069
30	Sciatic cut	
35	1·11	·067
45	0·77	·043

Exp. 2. Wt. 1·7 kgm.

0	70 c.c. Ringer's fluid injected into jugular vein	
15	7·38	·132
20	5·14	·132
25	Both symp. cut	
28	4·94	—
31	3·20	·135
35	2·93	·139
40	2·40	·133
42	Sciatic cut	
45	2·32	·138
50	2·26	·140
60	1·93	·130

Seven experiments were made on cats anæsthetised with urethane. The results are given in Table II, but the details of Exps. 5–7 which were similar to those of Exps. 1–4 are omitted. Two experiments were made on decerebrated cats to which after decerebration curari was given in about the amount required to paralyse somatic motor nerves (Table III). Exp. 1 in this table is inconclusive since the rate of blood flow was slow, but in Exp. 2 the result seems decisive. In none of the experiments did section of the sympathetic or subsequent section of the sciatic have any appreciable effect either on blood flow or on oxygen use. In these experiments there was the possibility that during the time taken to dissect the sympathetic, the tone of the muscles had been abolished. Experiments were then made on simple section of the sciatic, which in numerous cases has been shown to cause rise of temperature in the leg and flushing of the skin. The results are given in Table IV. The large increase of blood flow caused by the section indicates that the muscle was in an approximately normal state. The important result from my point of view was that the section did not decrease the oxygen use. In Exp. 1 a temporary increase was caused by contraction before the sciatic was cut, otherwise the oxygen use ran much the same course as in the experiments in Table I in which no nerves were cut. We may, I think, conclude that if the sympathetic has any tonic action on muscle, the tone does not lead to oxygen use. We may conclude also that the ordinary extensor tone kept up by the somatic nerves does not affect oxygen use. The latter conclusion is in harmony with the results obtained by Frank and Voit and by Roaf. Frank and Voit compared the total CO_2 production in dogs in a quiet state with that after giving curari in sufficient amount to paralyse somatic motor nerves, and found that the CO_2 production was either not appreciably affected, or was slightly increased by the paralysis. Roaf found that the CO_2 production in the extensor tone of decerebrate cats was not certainly greater than that after curari had been given. And H. Meyer and Fröhlich state that the strong tonic contraction caused in cats by tetanus toxin is accompanied by very slight muscular metabolism.

The fact that in Exp. 1, Table IV, and in one of the experiments of Table II, a movement of inconsiderable extent during the time of collecting a blood sample caused a definite increase of blood flow and of oxygen use, made it advisable to determine the extent of the increase in the conditions of the experiment, when the sciatic was stimulated. That stimulation of the sciatic causes, after a brief slowing, increase of blood flow was shown by Gaskell, the increase in oxygen use by

TABLE IV. Urethane. Sciatic cut.

Exp. 1. Wt. 2·4 kgm.				Exp. 2. Wt. 1·9 kgm.			
Hrs.	mins.		Blood flow per min. c.c.	O_2 use per min. c.c.	Hrs.	mins.		Blood flow per min. c.c.	O_2 use per min. c.c.
	0		3·57	·133		0		2·87	·099
	5	movement	4·49	·223		10		2·40	·102
	25	2 mins. after a movement	3·05	·182		40		2·87	·092
	58	Sciatic cut			1	9	Sciatic cut		
	59		9·33	·134	1	10		5·12	·108
1	4		4·72	·133	1	20		2·75	·091
1	7		3·83	·135	1	30		2·40	·096
1	59		5·29	·143	2	15		1·41	·100
2	6		5·22	·115	2	25		1·13	·085
2	15		2·09	·130	2	35		0·89	·072

TABLE V. Urethane. Sciatic stimulation.

Exp. 1. Wt. 3·0 kgm.				Exp. 2. Wt. 2·6 kgm.			
	0		9·00	·118	0		5·69	·206
	15		7·64	·113	10		3·96	·202
	40	Sciatic stim. 5 secs.			20	Sciatic cut		
	46		6·52	·190	1 5		9·33	·200
	58		6·08	·180	1 10		5·54	·207
1	7		6·00	·189	1 20		3·63	·207
2	0	Sciatic stim. 3 mins.	13·50	·938	2 1	Sciatic stim. 8 mins.	7·65	1·021
2	10		10·20	·689	2 6		3·91	·426
2	20		3·91	·236	2 18		2·43	·253

Verzár, and its long duration by Barcroft and Kato. The experiments in Table V show the very great effect caused by stimulating the sciatic for a few minutes in the conditions of my experiments, and Exp. 2 shows the contrast between the effect on oxygen use of the increase of blood flow caused by cutting the sciatic, and that caused by stimulating it.

Having failed to obtain any effect on section of the sympathetic, I tried the effect of stimulating it. The results are given in Table VI. It will be seen that in each case the stimulation caused marked decrease both in rate of blood flow and in oxygen use. In most cases the decrease in blood flow seems an adequate explanation of the decrease in oxygen use, for, as said above, if the oxygen supply falls below a certain point a decrease in oxygen use must occur. The results in Exp. 1 are less certainly explained in this way, they suggest a direct inhibitory action, but as the rate of blood flow was small for a cat weighing 3 kgm. it is probable that the rate had fallen considerably during the preliminary operation, and that the oxygen supply at the time of stimulation was close to the limit required for the ordinary needs of the muscles. It is I think clear that no metabolic change in the muscles leading to increased oxygen use is caused by stimulating the sympathetic. As the effect of

the sympathetic on the blood flow passes off there is a tendency for the oxygen use to become a trifle greater than it was before the stimulation, but the increase is not out of the range of experimental error, and the decrease of oxygen use caused by the stimulation is certainly not compensated by after increase.

TABLE VI. Urethane. Sympathetic stimulation.

Exp. 1. Wt. 3·1 kgm.

Hrs.	mins.		Blood flow per min. c.c.	O_2 use per min. c.c.
	0		3·00	·169
	5		2·87	·167
	35	Symp. cut		
	40		3·77	·162
	55		3·23	·162
1	5	Symp. stim. 1 min.	2·04	·131
1	10	Symp. stim. 1 min.	1·60	·068
1	15	Symp. stim. 1 min.	1·23	·042
1	30	Symp. stim. 1 min.	1·36	·045

Exp. 2. Wt. 2·7 kgm.

	0		11·00	·140
	10		9·00	·132
	15	Symp. cut		
	20		9·00	·131
	25		3·00	·135
	40	Symp. stim. 3 mins.	1·96	·046
	55	Symp. stim. 1 min.	2·40	·066
1	25		2·74	·130
1	35		3·20	·136

Exp. 3. Wt. 2·1 kgm.

Hrs.	mins.		Blood flow per min. c.c.	O_2 use per min. c.c.
	0		5·42	·090
	25	Symp. cut		
	35		2·65	·088
1	25	Symp. stim. 4 mins.	0·60	·035
1	40		1·60	·064
1	50	Symp. stim. 3 mins.	0·64	·044
2	10		1·20	·086
2	45		1·13	·092
2	55		1·00	·074
3	15		0·93	·058
3	25		0·74	·055
3	35		0·63	·053

Exp. 4. Wt. 3·0 kgm.

	0		3·34	·154
	10		2·81	·159
	20		2·11	·158
	25	Symp. cut		
	26		2·01	·150
	45	Symp. stim. 4 mins.	0·82	·071
1	0	Symp. stim. 1 min.	0·96	·082
1	10		1·32	·144
1	30		1·41	·159
1	50		1·22	·154
2	15		1·04	·130
2	30		0·90	·083

The results given in the preceding account as regards the rate of blood flow before and after nerve section leave some doubt as to their meaning. Section of the sciatic caused increase of blood flow when the sympathetic was intact but not when it had been cut. This suggested that the increased blood flow in the former case was due to cutting off tonic impulses passing by the sympathetic fibres of the sciatic—the usual explanation given of the rise of temperature and flushing caused by sciatic section. In favour of this suggestion were the facts (*a*) that the increased blood flow caused by sciatic section with intact sympathetic was not accompanied by increased oxygen use, so that it was not due to

contraction of the muscles, and (*b*) that after the sympathetic had been cut, the sciatic section did not cause increased blood flow so that it was not due to antidromic action. On the other hand section of the sympathetic itself did not cause increase of blood flow—this suggested that the sympathetic had no tonic action on the vessels of the muscles. The results would be harmonised if the increased blood flow caused by section of the sciatic were a reflex by way of the sympathetic, but it is difficult to see how this could be brought about. The only other explanation seems to be that the sympathetic tone was lost in the time taken to dissect the sympathetic, although the tonic action of the sympathetic on the blood vessels is generally held to be very persistent.

Two experiments were made on decerebrate and lightly curarised animals to see if the mechanical stimulation caused by cutting the sciatic, or the stronger stimulus caused by pinching, would cause increase of blood flow. Section of the sciatic caused no increase in blood flow. After cutting the sciatic, it was cut again lower down, and after this pinched once firmly with forceps. No effect was found, *i.e.* no evidence of antidromic action was obtained, but the experiments were not very satisfactory since the blood flow was slow, and the blood pressure was not taken.

A few experiments were made in which the venous blood was collected from the femoral vein, the pipette being inserted into the large saphenous vein (ext. saph.), so that part of the blood came from the skin. The oxygen use in these was not determined. In a urethanised animal, after the rate of flow had sunk from 6·5 c.c. to 2·6 a minute, stimulation of the central end of the sciatic of the opposite side caused a rise of blood pressure from 89 to 105, and a decrease of blood flow from 2·6 to 1·9 c.c. a minute. Section of the sciatic with the blood pressure at 94 caused an increase of blood flow to 7·2 c.c. a minute. In another experiment (rate of blood flow at the start 12·6 c.c.) section of the sympathetic had little if any effect; stimulation of the sympathetic caused a great decrease and stimulation of the sciatic a great increase of blood flow. In an experiment on a decerebrate curarised cat, section of the sciatic caused an increase of blood flow from 3 c.c. to 6 c.c. a minute; on pinching it later the blood flow fell from 2·23 c.c. to 1·36 c.c. a minute. The chief difference in the results of these experiments from those given previously is that section of the sciatic in the decerebrate-curarised animal caused increased blood flow.

The time at my disposal did not allow me to arrive at a definite conclusion as to the exact causes of the variation in the effect of nerve

section on blood flow. The central sympathetic tone is dependent on a good supply of oxygen to the central nervous system, and it is probable that the effect of nerve section is greatly influenced by the degree of local tone. It is known that when the skin is cold, section of the sympathetic may cause little if any flushing.

With regard to the main object of the experiments, I base my conclusion that the tonic action of the sympathetic on muscle, if it exists, does not cause an increase of oxygen use, mainly on the observations that oxygen was found to vary little with great variation in rate of blood flow, that section of the sciatic in the anæsthetised cat did not cause decrease of oyxgen use (Table IV) and that stimulation of the sympathetic caused decrease and not increase of oxygen use (Table VI).

Summary.

The results refer to the rate of blood flow, and oxygen exchange in the muscles and bones of the lower leg of the cat.

1. The rate of blood flow makes no difference to the rate of oxygen use except when the flow becomes very small.

2. Section of the sciatic when the sympathetic is intact causes in anæsthetised animals, in harmony with the experiments of others, an increase in rate of blood flow. The section does not cause decrease in oxygen use.

3. Stimulation of the peripheral end of the lumbar sympathetic causes great decrease both in rate of blood flow and in oxygen use; the latter is probably a consequence of the former.

4. I conclude that the sympathetic does not increase the oxygen use of the muscles, and, in agreement with Frank and Voit, that the ordinary (extensor) muscular tone kept up by somatic nerves does not increase oxygen use.

5. The rate of blood flow and oxygen use, both in anæsthetised and in decerebrate-curarised animals was found to be unaffected by section of the sympathetic nerves, and by section of the sciatic after the sympathetic nerves have been cut. The exact cause of the absence of effect in blood flow was not determined.

In conclusion I wish to express my thanks to Prof. Langley for his help in considering the results of the experiments, and to Mr Barcroft for his aid in the technique of oxygen estimation.

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EFFECTS ON THE FROG'S HEART OF VARYING THE ALKALINITY AND CALCIUM CONTENT OF THE PERFUSING FLUID.

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GASKELL(1) showed that a trace of caustic soda added to a solution perfusing the heart caused systolic standstill. This was shown by me some time ago to be dependent on the calcium salts in the perfusing solution(2). Mines(3) found that hearts contracted firmly when perfused with Ringer's fluid of C_H 10–12 (*p*.H, 12).

In the following paper I give some experiments on the effect of varying the C_H and the Ca concentration of the perfusing fluid.

The hearts were perfused through a cannula inserted into the inferior vena cava and the perfusing fluids were kept in Marriotte's bottles or inverted flasks with rubber tubing connections with the cannula. The change of perfusion from one fluid to another was effected by disconnecting one flask from the cannula and then connecting up the other. The hydrogen ion concentration of the perfusing fluids was determined by Sorensen's method.

A calcium-free stock solution was prepared having a composition of ·6 p.c. NaCl, ·03 p.c. KCl, ·01 p.c. $NaHCO_3$. To this was added as occasion required $CaCl_2$ to make ·0025 p.c. (= R.$\frac{1}{10}$Ca), or ·025 p.c. (= R), or ·15 p.c. (= R ·15 Ca).

Effect of decrease of Ca on increase of alkalinity. When a heart is perfused with Ringer's fluid deficient in Ca, the beats as is known decrease. When the decrease is established, an increase in the alkalinity, *e.g.* from *p*.H 7·5 to *p*.H 9 causes a slight brief decrease in the force of the ventricular beat, followed by a slow increase. On replacing the original solution, there is a slight brief increase in the force of the ventricular beat, followed by a slow decrease. These effects are shown in the three experiments in Fig. 1 in which the heart was first perfused for about an hour with Ringer's fluid deficient in Ca (R.$\frac{1}{10}$Ca).

It may be noted that the primary decrease in the height of the ventricle beat on increasing the alkalinity is equal to the primary increase on restoring the original alkalinity. These primary effects are I think due to an action on a mechanism different from that which is acted on in producing the slow subsequent decrease.

Perfusion of the heart with the solution deficient in Ca, allows the alkalinity to be increased beyond the limit given by Mines as that which stops the heart in systole, without its producing this effect. This is shown in Fig. 1 *C*; it will be seen that with an alkalinity of *p*.H 12, the heart after a brief slight depression gradually increased in force and beat much more strongly than with the original less alkaline solution. The increase in force of beat I attribute not to a direct effect of the alkali, but to the change it produces enabling the calcium to act more strongly.

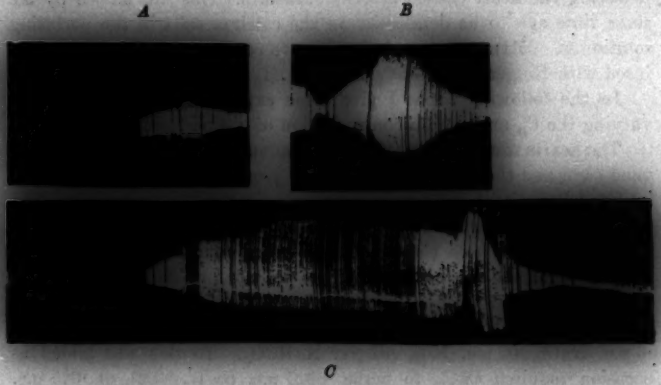

Fig. 1. Hearts perfused with Ringer's fluid deficient in Ca (·0025 p.c.) and of *p*.H 7·5; then alkalinity first increased and soon after restored to the original amount; *A*, *p*.H = 9; *B*, *p*.H increased but indetermined; *C*, *p*.H = 12.

Effect of increase of Ca on increase of alkalinity. When the heart has been perfused with a Ringer's fluid containing excess of Ca (·15 p.c.), a certain degree of increase of alkalinity (*p*.H 7·5 to *p*.H 11) has very little effect, Fig. 2 *A*. There is a slight depression after an hour's perfusion (cp. Fig. 2 interval between *A* and *B*). On then perfusing with a fluid containing the same constituents except Ca, the heart soon stops in diastole (Fig. 2 *B*, first arrow 0 Ca), and on re-addition of ·15 p.c. Ca, the heart passes into a state of tonic contraction with beats of small excursion (Fig. 2 *B*, second arrow). These results show that Ca antagonises the depressive action of the alkali, and prevents the alkali from producing the cardiac change in virtue of which the action of Ca is increased.

Effect of alkali on the hypodynamic heart. Prolonged perfusion of the heart with ordinary Ringer's fluid causes as is known decrease in the force of the beat. In this state, the depression caused by increase of alkali is much greater than in the fresh heart, and it may cause primary diastole before the heart passes into systole. Traces of adrenin restore the heart's capacity to resist this depression.

It has been shown by Dale and Thacker that the optimal hydrogen ion concentration varies in different parts of the heart. The experiments I have given above show that it varies also with the condition of heart, especially as regards the calcium content of the fluid bathing it. It is possible then that in pathological conditions of the heart the optimal hydrogen ion concentration is different from that in the normal state.

Fig. 2. Variation in alkali and of $CaCl_2$ (see text). *M*, readjustment of lever. *PR*, increase perfusion pressure. At third arrow, perfusion with R ·15 p.c. and *p*.H of 8.

Summary.

In perfusion experiments on the frog's heart, the effect of alkali depends within certain limits on the amount of calcium. Similarly the effect of calcium depends on the amount of alkali. After perfusion with a Ringer's fluid deficient in calcium and having a *p*.H of 7·5, the heart after a primary slight depression will beat strongly, although the alkalinity is increased to a *p*.H of 12. Increase of calcium prevents both the primary depression and the subsequent augmentation.

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THE REGULATION OF THE WATER CONTENT OF THE HUMAN ORGANISM.

By EDWARD F. ADOLPH, Ph.D.

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Methods. In the following experiments I have attempted to define some of the conditions which determine the amount of water in the body.

Intravenous injections present very unusual and drastic conditions to organs of regulation, particularly to the kidneys. The experiments now to be described were designed to exhibit the normal adjustments of the body fluids, and were therefore made by introducing water and other substances into the circulation from the alimentary tract, or curtailing their introduction.

The analytical methods employed were as follows: Urine volumes were measured to an accuracy of 3 p.c. in graduated cylinders. Specific gravity was determined with a hydrometer spindle after the urine had cooled to the temperature which the hydrometer required. Chlorides were estimated by the modified Volhard method of Harvey(1), urea by the original titration urease method of Marshall(2), ammonia by formol titration using phenolphthalein as indicator (Malfatti(3)), and acid by titration to neutral red after the manner of Henderson and Palmer(4). Actual acidity was measured by comparison of undiluted urine to the phosphate standards of Sörensen(5), using cochineal tincture as the indicator in the usual acid range. For hæmoglobin estimations the original Haldane(6) hæmoglobinometer was employed. Alveolar air was analysed on the small Haldane analyser, the Haldane-Priestley(7) method being used for sampling. Body-weight was measured to ·01 kgm. upon a large balance sensitive to ·1 gm.

1. *Adding water to the body.*

In attempting to increase the water content, water was ingested either alone, or with various salts or urea, or following the salts. Water drinking has been studied by Haldane and Priestley(8, 9). By drinking 5500 c.c. in three hours, J. G. P. ingested water much faster than the kidneys excreted it, for the limit of excretion was 800 c.c. an hour. No dilution of hæmoglobin in the blood was found, though excretion was maintained at the maximum rate for more than four hours after drinking had ceased. Similar experiments done on myself show also that the hæmoglobin of the blood is constant during water diuresis. Any storing

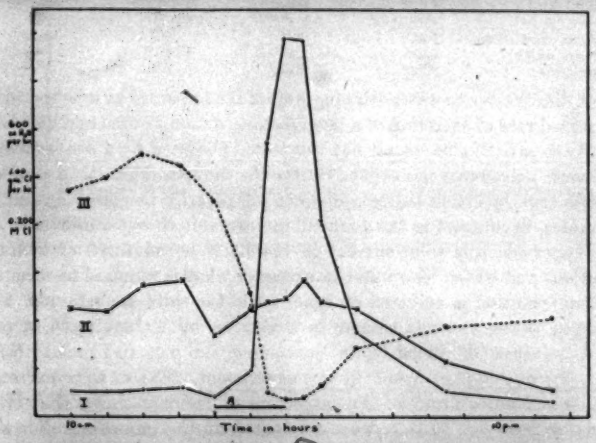

Fig. 1. Urine excretion after drinking 1500 c.c. water at A, Oct. 14. I. Rate of water excretion. II. Rate of chloride excretion. III. Concentration of chloride.

of water is merely a temporary makeshift supplementary to the activities of the kidneys, since if the body is previously satiated normally with water, the same amount will be excreted in the course of the diuresis as was taken in. This is illustrated by the following two experiments (Fig. 1):

1. Drank 1500 c.c. water in two hours. In four hours excreted 1550 c.c. Average rate of excretion in four preceding hours 54 c.c., in two succeeding hours 57 c.c. Maximum rate of excretion 790 c.c. an hour.

2. Drank 2750 c.c. water in five hours. In seven hours excreted 2900 c.c. Average rate of excretion in two preceding hours 45 c.c., and in six succeeding hours 43 c.c. Maximum rate of excretion 920 c.c. an hour.

It is evident that the water content of the blood and tissues is not changed by the taking of water alone. Erroneous conclusions may be drawn from similar experiments in case the body has not been already supplied with water up to its physiological normal, or if the intake is so slow that some of the water escapes by other channels than the kidneys. It might be supposed that the diuresis due to water would wash from the body some of the substances to which it is physically related in the organism. That this is not so, is shown by the fact that the total solids eliminated in the 24 hours during which copious water is drunk are approximately the same as in 24 hours of complete thirst.

	Water c.c.	Acid c.c. *N*/10	Ammonia c.c. *N*/10	Chloride gm.
Without extra water	1680	294	561	9·36
3000 c.c. water	4550	247	485	7·63
30 gm. urea	2390	327	706	7·06

A diuresis due to water drinking is often accompanied by a temporarily increased rate of excretion of a few substances such as urea and chloride, as Marshall(10) also found, but this is compensated by a lessened rate for these substances immediately after the diuresis (Fig. 1). A diuresis due to urea ingestion led to a distinct temporarily increased output of chlorides, in contrast to the doubtful increase due to water diuresis.

Hypotonic salt solutions act in the body as mixtures of isotonic solutions and water. The water in excess of what is required to form an isotonic solution is excreted at once, while the salts are retained. The solution taken was one similar to that used by Priestley(9), which is a mixture of blood salts containing 0·6 p.c. (0·1 molar) NaCl (Fig. 2); of 2000 c.c. drunk in my experiment, 285 c.c. were excreted during the water diuresis. An inspection of his data shows that after drinking 2000 c.c., 715 c.c. were eliminated during immediate diuresis; while after drinking 3000 c.c., 1420 c.c. were eliminated, in excess of the regular rate of excretion of water as given by the fore and after periods. While it is possible that the subject J. G. P. excretes isotonic salt solution in a rapid diuresis, it is evident that he did not immediately eliminate half of the amount drunk.

Approximately isotonic salt solutions behave in a manner strikingly different from those of other concentrations. One per cent. NaCl was drunk, either slowly or in a dose of 3000 c.c. in an hour. When taken slowly it was eliminated in an increased urine volume which did not exceed 100 c.c. an hour (Fig. 3). When 3000 c.c. were taken in an hour the diuresis mounted to 300 c.c. an hour, and remained above 100 c.c. an hour for more than 24 hours. During this period the chlorides were

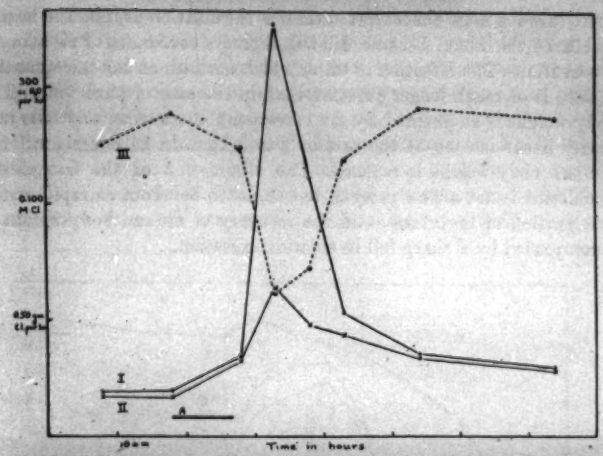

Fig. 2. Urine excretion after drinking at A 2000 c.c. of Locke's solution, which was slightly hypotonic, Jan. I. Rate of water excretion. II. Rate of chloride excretion. III. Concentration of chloride.

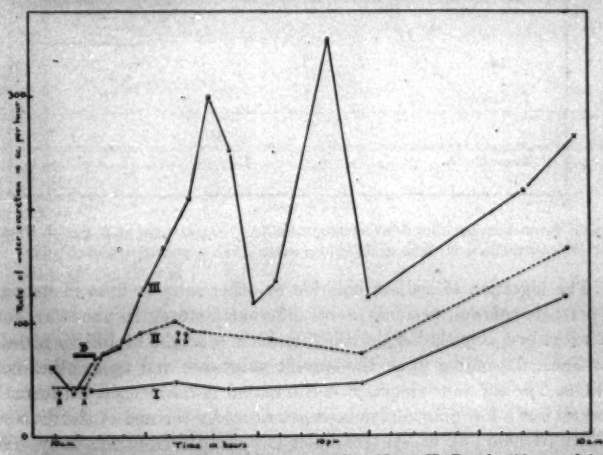

Fig. 3. Rates of water excretion. I. Normal day, Nov. II. Drank 500 c.c. of 1 p.c. NaCl solution at each of four points A, Nov. III. Drank 3000 c.c. of one p.c. NaCl solution at B, Jan.

nearly always more concentrated than in the solution drunk. The hæmoglobin of the blood became diluted slightly, confirming Priestley's observations. The retention of water which contains an isotonic quantity of salt, is of much longer duration than in the case of pure water. The body-weight is augmented by the corresponding amount, and falls in a nearly linear fashion at the rate of 1 to 2 kgm. in 24 hours until the normal body-weight is reached. The elimination of the last excess of chloride is not a slow process, but seems to be about as rapid as the first portion of the excess, and the recovery of normal body-weight is accompanied by a sharp fall in chloride excretion.

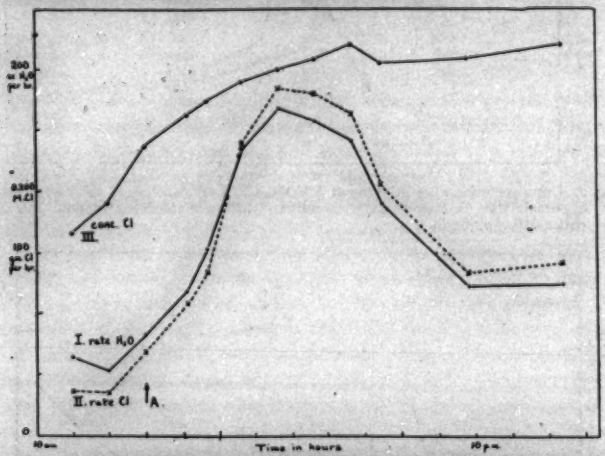

Fig. 4. Urine excretion after drinking 30 gm. NaCl in 300 c.c. water at A, Oct. I. Rate of water excretion. II. Rate of chloride excretion. III. Concentration of chloride.

The ingestion of sodium chloride or other salts or urea in strongly hypertonic solution presents a still different picture. In about an hour an excretory diuresis begins which reaches a height of 100 to 200 c.c. per hour, depending upon the specific substance and upon other conditions. The substance ingested is eliminated in a concentrated form, so that all but a few grams have been got rid of by the end of the diuresis. Within 24 hours all of the remainder has escaped from the body, over and above the normal daily amount. The maximum water output is thus often above that for isotonic elimination and the elimination of both

water and salt is much more rapid (Fig. 4). The hæmoglobin of the blood was found to be concentrated slightly during the period of absorption from the intestine. It might be supposed that salt introduced in the proper highly hypertonic concentration will prevent diuresis by holding the water in the body, so that there is none to spare for a diuresis. No such phenomenon has been found. The organism is evidently able to distinguish any mixture which is foreign to the complete composition of each tissue substance. The most successful retention so far found for water given by mouth has resulted from taking 1 p.c. NaCl solution.

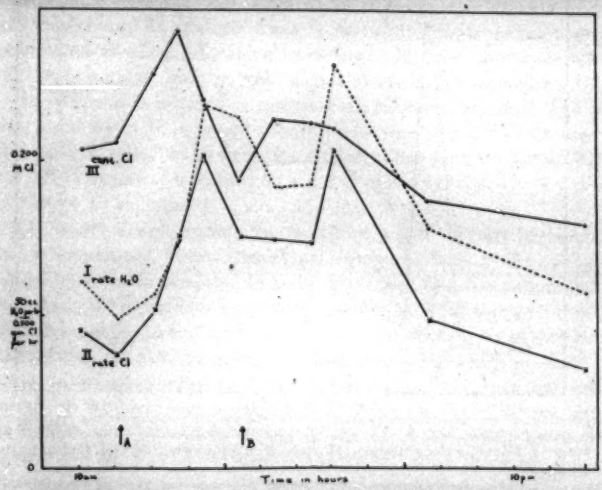

Fig. 5. Urine excretion, Nov. At A drank 10 gm. NaCl in 100 c.c. water; at B drank 750 c.c. water. I. Rate of water excretion. II. Rate of chloride excretion. III. Concentration of chloride.

In one experiment an attempt was made to stop a diuresis initiated through taking strongly hypertonic sodium chloride, by ingesting enough water to make the remaining salt isotonic. Far from inhibiting the diuresis, a large quantity of water was immediately excreted, carrying with it salt in the high concentration attained in the primary diuresis (Fig. 5). The entire diuresis was thus much shortened by rendering water available for carrying away the salt. The converse experiment was then carried out. As much water was taken as could be drunk at one time,

and after a diuresis of 215 c.c. per hour had been attained 15 gm. of sodium chloride were taken. Within an hour the water elimination was completely inhibited; but a salt diuresis occurred which presumably reduced the salt rapidly to an isotonic amount (Fig. 6). The remainder was then eliminated very slowly during the next 24 hours just as though 1 p.c. NaCl had been ingested. The general conclusion is that water is held in the body for a short time if it is absorbed simultaneously with a quantity of salt which bears some quantitative relation to it. If either

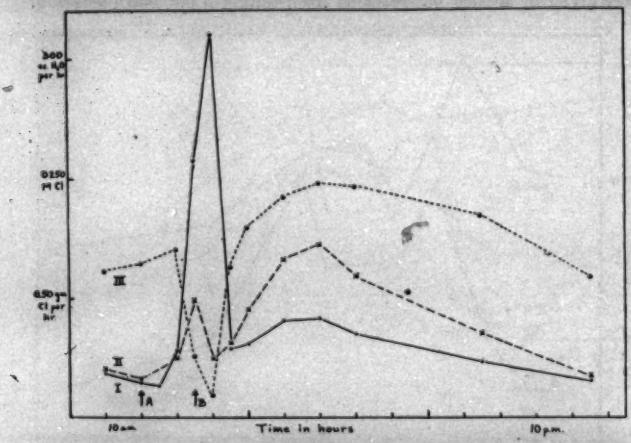

Fig. 6. Urine excretion, Jan. At A drank 1300 c.c. water; at B drank 15 gm. NaCl in 80 c.c. water. I. Rate of water excretion. II. Rate of chloride excretion. III. Concentration of chloride.

is absorbed in excess, the excess is eliminated immediately through the kidneys.

The experiments upon the drinking of water and neutral solutions lead to one generalisation of importance. It is that water held by the organism is distinct from water freshly absorbed from the alimentary tract. The evidence for this structural nature of body water is as follows:

(1) Water is mobilised for excretion at a rate which does not vary 10 p.c. when bodily activities are constant. This water is in excess of that which the solids of urine require for elimination in limiting concentration, which Ambard and Papin(11) term the obligatory water

excretion. The mobilisation is so regulated that the water demands excretion even in addition to an excessive supply of ingested water.

(2) During a period of 24 hours after the body has been well supplied with water, water taken alone will all be excreted at once. In the previous hours of the period the body has lost water at a constant rate of about 45 c.c. an hour, and one might suppose that some of the newly ingested water would be retained to compensate this loss. This is not so; one may infer that the water excreted was wholly superfluous. Thus, Gerhartz(12) showed that water alone will not reinstate the loss of body-weight which occurs during exercise. But when water is taken with other anabolic materials, it may be retained. There is no call for tissue water until the tissue quota of other substances is restored. In brief, the water taken with meals is alone destined to be a part of the body's structure.

(3) When salt is taken it causes a diuresis which carries into the urine more water than would otherwise be eliminated. The diuresis is followed by a period when water is excreted at less than the normal rate. Evidently the rate of mobilisation is not altogether changed by a sudden stimulation to excretion.

(4) Water drunk during the course of a salt diuresis hurries the diuresis immensely. Water already present in the body is not available so quickly as water unrelated to body substance. The elimination of salt proceeds as if the kidneys had set themselves to finish the task already begun in response to the sudden supply of salt; whereas when the water is taken with the salt, no sudden elimination takes place.

(5) In an experiment urea was ingested at a time when the water content of the body was much reduced. With unusual slowness water was mobilised to eliminate a part of the urea; but when water was ingested a great rise in the rate of urea elimination occurred, though the water in the tissue was still below the physiological normal.

The distinction between the excretion of water already mobilised and that which is not available for excretion has its counterpart in the salts. Ingested sodium chloride is eliminated very quickly and almost quantitatively in so far as it is taken without water. But as much of this salt as will form an isotonic solution with water already mobilised, is not eliminated quickly. Again, salt tends to be excreted at a constant rate, and is mobilised for this excretion ahead of time, so that a sudden diuretic flow caused by urea ingestion will drag out a small amount of this mobilised salt, for which the body will tend to compensate by a lag in the salt excretion after the diuresis is over. If salt intake is stopped by instituting a chloride-free diet, the excretion of salt goes on for more

than 24 hours unaltered in rate. Yet no amount of water taken meanwhile will hasten by more than 50 p.c. the salt excretion. Three experiments showed that when a large amount of salt was excreted in the sweat, the urinary output of salt almost vanished. It becomes clear that there is some good reason for the remarkable constancy with which all urine constituents are excreted. On any other view it is difficult to understand why the body excretes salt at all, except when it receives some. One is led, therefore, to think of water and salts as arising in the urine in two separate ways. In one case water or salt in excess of the body's needs is promptly taken by the kidneys; in the other case water and salt are supplied at a fairly constant rate through liberation from body tissues. There is a similarity in these substances to the urea of the urine, which Folin(13) found was supplied for excretion in sudden quantities after an excessive protein meal, and in a more constant quantity at all other times.

Salts or urea in any combination are manifestly unable to bring about more than a transient retention of water in the organism. The whole science of nutrition serves to show further that no combination of proteins, carbohydrates, and fats will of themselves lead to a corresponding manufacture of tissues with their corresponding need for water. Carbohydrates may temporarily be deposited in the body, but these are no more permanent additions than salts; and have been shown by Benedict and Milner(14) to hold water with them; they are stored, not incorporated. Fats also may be regarded conceivably as representing permanent additions to the organism's structure, but they have no water associated with them, and in fact are shown by some evidence to displace water. Salts will assist in the temporary retention of water, and to a smaller extent water will help to hold salts in the body. But neither will become an integral addition to the body's substance merely from their presence.

2. *Diminishing the water content of the body.*

Reduction of the water content is possible, and is of great interest. In hot climates the mere stoppage of water ingestion leads to extremely rapid dehydration of the body. Thus Hunt(15) has shown that the minimal loss of Europeans leading an active life in Central India was 13 litres a day. In cold weather the reduction of body water is much more difficult, especially when the humidity is high.

The first experiment lasted four days, early in November. The food was kept as nearly as possible like that ordinarily taken, both in amount and in proportions, but was selected from nutrients having small water

content—chiefly meat, bread, butter, jam, cake, and sugar. Very little exercise was taken, corresponding to the normal custom of the subject, so that it took four days to reduce the body-weight from 70 to 67 kgm. (Fig. 7). On the fifth day, 15 gm. of NaCl were ingested, resulting in a diuresis which further reduced the weight by ·6 kgm. Water was then drunk in moderate amounts, and in 24 hours 3500 c.c. were taken, in addition to hydrated foods, before the first water diuresis occurred, indicating that the body was now normally supplied. The body-weight rose to 71 kgm. and stayed at this mark. Thus the body was not only

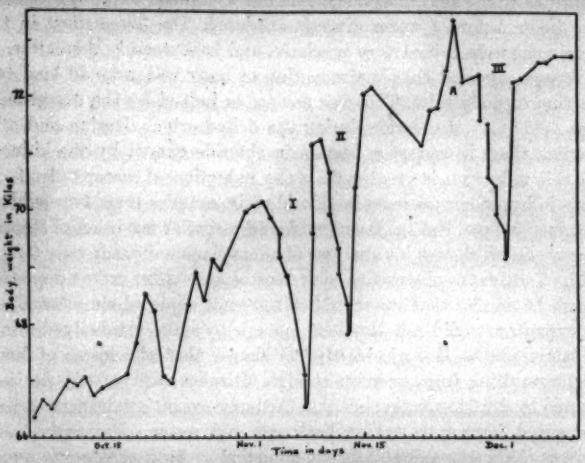

Fig. 7. Curve of body-weight. E. F. A. 1920. I, II, III designate the three dehydration experiments. At A 2000 c.c. of one p.c. NaCl were drunk.

capable of taking up all the water which had been lost, but also a small amount of excess, due to the increase of other materials proportional to the general curve of growth in weight over this entire period.

In a second experiment not only was the water intake stopped, but sweating was attempted. The respiration chamber was heated with moist heat so that the wet bulb temperature was 34° C. In two hours of active sweating only 0·65 kgm. was lost, which was much less than anticipated. However, an accidental infectious diarrhœa helped somewhat to reduce the body's water reserves, and in three days the weight dropped from 71 kgm. to 68·3 kgm. On the third day 30 gm. of urea were ingested,

resulting in a diuresis, which took away 0·6 kgm. additional water. Immediately after the diuresis water was ingested, and the body-weight rose 4 kgm. in 24 hours before constancy was attained.

In a third experiment exercise was employed to reduce the water reserve in addition to sweating and thirsting. On the third day 0·8 kgm. was lost by exercise, on the second day 0·9 kgm. was lost in two hours of active sweating. On the third day 0·6 kgm., and on the fourth 0·7 kgm., were lost by the diuresis resulting from the ingestion of 30 gm. and of 45 gm. respectively of urea. Then water was taken in 500 c.c. quantities at one hour intervals for six doses, and the next day two doses were taken before a water diuresis appeared. The 3 kgm. lost in this experiment were gained very precisely, and held steadily thereafter.

It was expected that the reduction of body water would lead to a rejection of body salts. This was not so, as judged by the excretion of total solids and of chloride during the dehydration. During and after sweating there is a great reduction in chloride output by the kidneys, and this reduction is greater than the reduction of water output, for sweat is hypotonic as regards chloride (six samples from two subjects contained 0·6 p.c. NaCl). Hunt(15) found a similar retention of chloride after sweating, though his analyses of sweat show uniformly only 0·2 p.c. NaCl. Tobler(16) observed a slow loss of salts after water depletion, though he admits that water and salt are not depleted simultaneously. My experiments did not last long enough to make the body diminish its salt content. Cohnheim(17) has shown that the losses of body-weight resulting from exercise at high altitudes can be only partially restored by drinking water; but that the body-weight can be permanently augmented further by taking both salt and water. Evidently in his subjects there was a simultaneous depletion of both substances due to the sweating which accompanied the exercise. One may say that the kidneys are more discriminating than the sweat glands; the latter excrete a fluid of constant composition for a given unacclimatised individual, while the former can secrete a fluid of almost any composition which the internal environment of the body calls for. While salts may be saved when water is lost, the converse does not occur to any great extent. The experiments of Rosemann(18) indicate that in chloride-free diet and in starvation, water is lost even in higher proportion than the chloride and urea losses.

The above experiments in dehydration show that the water content of the human organism may be reduced by various means, whose comparative rapidity is given in Table I. This rapidity varies widely with

TABLE I. Rates of water loss over short periods of time.

Subject	Conditions	Total water loss kgm. per hour	Loss in urine	Loss in sweat
E. F. A.	Rest—Nov.—Oxford	·10	·05	·04
72 kgm.	Exercise on ergometer	·17	·07	·08
	Diuresis due to salt	·18	·13	·04
	Sweating at 34° wet bulb	·32	·02	·29
	Bath in water at 39°	·75	·24	·50
H. C. B.	Rest—Jan.—Oxford	·14	·05	·08
61 kgm.	Sweating at 34° wet bulb	·68	·04	·63
	Bath in water at 39°	1·30	·40	·89

individuals. The experiments also show that the reduction of water content does not lead to the depletion of salts or any other tissue constituents. The body-weight is completely restored by drinking water. Moreover, it is shown that a moderate deficiency of water does not constitute a limiting factor for growth in weight. Fig. 7 gives the curve of body-weight over a period of two months. The first six weeks of this period constituted a time of phenomenal growth in an adult subject; growth at the rate of 1 kgm. a week. Such a "growth" is the annual occurrence with this subject. In the two dehydration periods growth evidently proceeded at the same rate as previously, for a proportionate gain appeared upon supplying water. In the third dehydration experiment growth had ceased, and here there was no advance on the former weight when water was taken *ad libitum*.

The impression of numerous authors seems to be that there is a "reserve" in the body of "free" water. No single fact in my observations lends itself to support this view, which pictures a store of water similar to that of glycogen and fat. But the present data indicate that water is analogous to amino nitrogen in the body. None is stored; but the supply of circulating water is kept constant, even at the expense of tissue structure, just as the amino acids and glucose are kept constant during starvation.

3. *The measurement of body hydration.*

The most familiar indication of the body's ability to acquire more water than it has, is given by the sensation of thirst. This sensation may be reinforced in extreme thirst by more generalised restlessness.

Rapid changes in body hydration may be measured as a function of the blood's so-called osmotic pressure. In this way Priestley(9) has been able to detect a reduction in the conductivity of blood during the absorption of water. When isotonic salt solution is drunk I have found, as he did, a decrease in hæmoglobin concentration. When dehydration

of the body occurs, the hæmoglobin increases. Thus in the three experiments mentioned above where 3·5 litres of water were lost in each case, the relative amounts of hæmoglobin rose from 100 to 110, 109, and 107 respectively. In other words, a decrease of 5 p.c. in weight diminished the proportion of water in the blood by 9 p.c.; the water loss is distributed throughout the body, but unequally. Greater changes in blood concentration may be caused by more sudden losses. In 1·5 hours in the hot water bath, E. F. A. lost 1·3 kgm. of water, and suffered a concentration of hæmoglobin of 5 p.c. Dr Bazett, who kindly gave me the results of a similar bath experiment, found his hæmoglobin to increase 15 p.c. in losing 2·3 kgm. in 1·5 hours, but only 2 p.c. while losing the same amount of water in three hours in the sweating chamber. Evidently the loss can be so rapid that a proper equilibrium is not maintained between blood and other tissues, and this indicates something of the time required for water mobilisation. Hunt(15) found no change in blood concentration in any of three subjects who each lost 2·3 kgm. in 2·5 hours in a Turkish bath.

Variations in the body-weight of the individual may be representative of water content, when certain limitations are understood. Roughly, if a change of weight represents variation in water content, the body must return to its normal weight, when standard conditions are again supplied. This is illustrated in the curve of body-weight (Fig. 7). The weighings were made at the same time each day just after urination and defecation, so that changes during the day's routine were avoided.

The ease of sweating might conceivably furnish a quantitative measure of the body water for any single person. The few experiments which have been done indicate no reduced rate of sweating under standardised conditions when the body is dehydrated. The composition of sweat in plethora and in thirst might be expected to vary. A few observations made upon this point showed a remarkable uniformity of composition; the concentrations of chloride and of alkali seemed to be the same in all samples of sweat from the same individual. Hunt(15) also searched for a difference in chloride concentration under various conditions, but found none.

Many authors have noted a relation between water reserve and fever. In dehydration I have not found a poorer regulation of temperature than in plethora; the limits to the body's use of water for regulating temperature were not reached.

The rate of water excretion at times when there are no digestive or other unusual activities, is precisely correlated with the level of body

water. During laboratory activities and at the hydration point, the average excretion rate is 45 c.c. per hour for E. F. A. Upon the initiation of a thirst regime the excretion falls off rapidly at first, then more and more slowly, and has reached the low level of 17 c.c. per hour. There is probably a fairly simple mathematical relation between the rate of excretion and the body's water content. A possible exception to this relation exists in the fact that the sudden absorption of "free" water into the thirsting body may lead to a diuretic excretion of a store of solids remaining to be excreted, before the body is satiated. This occurred in an experiment where 2000 c.c. of water were ingested after a dehydration of 4 kgm., and an excess of urea was present.

For a single individual the hourly water excretion is not the same on two days unless the physical and mental activities are the same. It is not constant throughout a single day; meals greatly inhibit water and chloride excretion. For the subject E. F. A. the normal curve of water excretion has one very salient feature, namely, a very high rate of nocturnal excretion. This is a very unusual peculiarity, and as far as is known has always existed, and is accompanied by no other feature which could possibly be regarded as pathological. The bladder is correlated to the extent of holding with comfort the large nocturnal volume, and is known to hold at least 1700 c.c. One of the common tests of nephritis is based on the fact that most persons secrete twice as much urine during the day as during an equal number of night hours. The average of 19 nocturnal excretions for E. F. A. in only 9¼ hours is 54 p.c. of the 24 hour excretion. This unusual feature of excretion does not detract from the value of the subject's experiments. The nocturnal urine averages 120 c.c. per hour during periods of adequate water intake; the day-time excretion, whether walking, standing or sitting, is about 45 c.c. per hour. A nocturnal excretion may be obtained at any time by lying down. The nocturnal urine is very dilute, having a specific gravity of about 1·009 as compared to 1·002 for day urine. But in chloride there is very little change in rate of excretion, so that in general all the solids are merely diluted, maintaining the rates characteristic of the day-time period. If water intake is limited, the nocturnal secretion is abruptly reduced to the day-time rate, keeping the output of solids constant. This atypical feature therefore furnishes in itself a useful indicator of bodily water plethora. If an excess of isotonic sodium chloride has been ingested, the nocturnal volume increases slightly, while not getting rid of the salt at more than the corresponding day-time rate.

The diuresis which arises as a response to the ingestion of water,

urea, or sodium chloride is a delicate indicator of the body's water supply. The chief attention in these experiments has been turned to the production of urine over half-hour or hour periods during the excretion of abnormal amounts of these three substances. If water is taken, after food has been supplied without water, or after sweating or salt diuresis, it is not excreted until that amount, which is a function of the nature of the newly acquired body substance, has been retained. The amount retained can readily be found. As an example, it was desired to find whether the high rate of nocturnal excretion gave rise to a deficit in

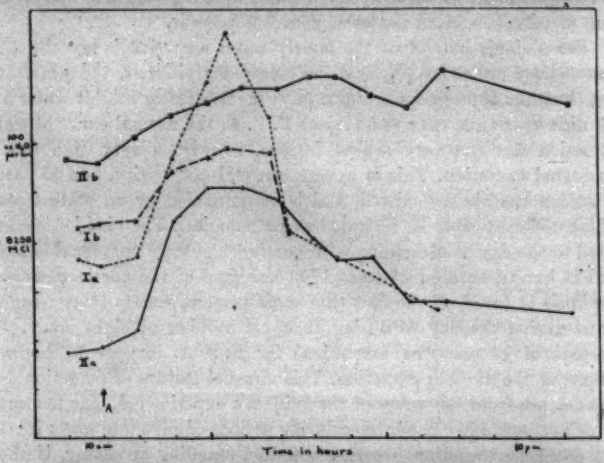

Fig. 8. Urine excretion after drinking 15 gm. NaCl in 150 c.c. water at A. I. Body fully hydrated. *a*. Rate of water excretion. *b*. Chloride concentration. II. Body dehydrated 5 p.c. of its weight. *a*. Rate of water excretion. *b*. Chloride concentration.

body water upon getting up. After emptying the bladder of 1200 c.c. excreted in nine night hours, 500 c.c. of water were drunk. While a slight diuresis resulted, only about 100 c.c. was excreted in addition to the normal rate. This demonstrates that when lying down the subject rejects water which the active body holds. Urea and sodium chloride produce diureses which have approximately the same characters. The chloride excretion is shown in Fig. 4. But when the body is dehydrated the picture of the diuresis aroused by a dose of urea or salt is very different (Figs. 8 and 9). In dehydration (1) the maximum rate of water output is

much less, (2) this maximum is reached more quickly, (3) the rate declines more gradually, (4) the diuresis lasts longer so that only $\frac{2}{3}$ as much solute is eliminated in 12 hours, (5) the solute attains a higher concentration, (6) it attains the maximal concentration more slowly and long after the highest water output, (7) in the single case of chloride diuresis, the water output following the diuresis does not go below normal as it does when the body has a normal water content, for it is already at the obligatory rate (Fig. 9). In my experiments dehydration never reached

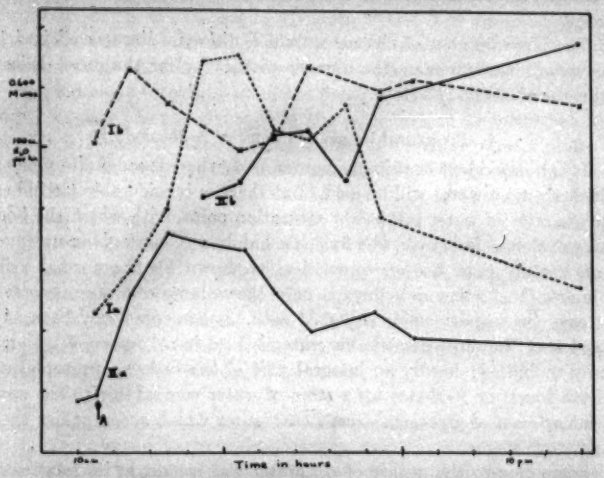

Fig. 9. Urine excretion after drinking 30 gm. urea in 120 c.c. water at A. I. Body fully hydrated. *a*. Rate of water excretion. *b*. Urea concentration. II. Body dehydrated 5 p.c. of its weight. *a*. Rate of water excretion. *b*. Urea concentration.

an extent such that a diuresis failed to result as a response to urea or salt ingestion. But the efficiency of the diuresis, as measured by the total amount of the excessive substance eliminated during it, declined progressively. On the other hand, as measured by the concentration of the substance got rid of the efficiency increased.

If the increased rate of water excretion in the case of E. F. A. when lying down indicates that the body is rendered super-hydrated for the time being, the response to a diuretic salt should be smaller. To test this point the subject slept, one night, in the laboratory, and stayed

in bed all the next day. Some time after waking a dose of 30 gm. of NaCl was taken, and the diuretic picture, compared to the normal curve in Fig. 4, was as follows: (1) an initial inhibition of the regular water excretion of 200 c.c. an hour, (2) a subsequent increase in water excretion which attained only 270 c.c. an hour, (3) a later fall to 140 c.c. an hour, (4) the total elimination of chloride was much more rapid than normally, since little disturbance of water mobilisation was required, (5) the maximum concentration of chloride was just as high as in response to the same dose given under normal circumstances, and was attained just as slowly.

The large number of characters which differ in diureses elicited by the same means, renders the diuretic picture by far the most delicate indicator of the body's state.

4. *Structural nature of the water in the body.*

It is now evident that in the human body there is a definite point at which no more water will be held. But there is considerable latitude in the quantity of water just below saturation point, with which the body can get along. Moreover, the first few hundred c.c. are given up much more readily than further quantities, as shown by the normal urine volumes. In further dehydration only the volume of water necessary to carry the urinary solids is parted with. It has commonly been supposed that the most dispensable water is held in a "reserve" which is easily mobilised; hardly an integral part of the body's "protoplasm." I have found no evidence for a store of water comparable to the store of fat or even of glycogen, except that water which accompanies these temporary stores.

Some clue to the nature of the water was sought in its relation to the acid-base equilibrium. Throughout the experiments I have searched diligently for such a relation, measuring the rate of acid excretion, the rate of ammonia excretion, and the hydrogen ion-concentration of the urine. The latter of course varies with the dilution of the urine, an important factor during diuresis. Numerous data in these experiments seem to show that acid excretion drops when water excretion rises, but many instances contradict this rule. It seems impossible to draw any conclusion in the matter, and a conclusion is not, I believe, of fundamental importance. The statements of other workers upon the relation of water excretion to acid excretion are sufficiently varied to indicate that when a relation is observed it is due to some other factor.

Another way of attacking the question is to see whether the pro-

duction of acidosis will give a greater water excretion than alkalosis will. Experiments in which acidosis was produced in the manner recently described by J. B. S. Haldane, by ingestion of ammonium chloride, gave about the same amount of diuresis as experiments with ingesting sodium bicarbonate. The classical experiments of Bischoff and Voit (1860)(19) on dogs showed that a change from carbohydrate to protein diet led to loss of body-weight. This has been interpreted as an effect of the acid diet upon the water "reserve." But Benedict and Milner's(14) experiments on man point to the conclusion that the loss of water is due solely to the exhaustion of glycogen stores. The actual structural constituents of the body are related to water content. This is shown by the remarkable constancy in the water percentage of tissues. If glycogen is regarded as an integral part of the body, a certain quantity of water, which is related to the glycogen in the same fashion as salt in isotonic solution is related to water, is also a part of the body. But I prefer to regard both as temporary additions to the body. A carbohydrate-free diet reduces the water content in the same way as the elimination of an excess of salt.

The experiments so far carried out show that no single substance will change the body's regulation of water content, though all of them are necessary for its maintenance. Water is lost upon a diet free of chloride for the same reason that it is lost on a nitrogen-free diet(18). The water is related to the whole of the body's constituents; it is a part of the body's almost immutable structure.

Summary.

1. The addition to the body of water, alone or accompanied by various salts or urea, is not permanent. The solution retained for the greatest length of time is 1 p.c. sodium chloride.

2. Water may be abstracted from the body by a large variety of methods, several of which have been studied comparatively. When no other constituent has been abstracted meanwhile, the body-weight is completely restored by drinking water. Depletion in water does not inhibit the process of growth.

3. The excretion of water, chloride, and urea during a diuresis due to any one of them is by far the most accurate indication so far studied whether the water of the body is normal in quantity.

4. The water content of the whole organism is independent of every other single substance. The content may be raised by introducing a temporary store of salt or carbohydrate; this water is not a part of the

essential structure of the body. Other than this, no evidence has been found for a reserve of "free" water, and in thirst the excretion of water is kept up at the expense of water from the tissues themselves.

I wish to express my gratitude to Dr J. S. Haldane for the opportunity of working in his laboratory and of sharing daily his intellectual activities. His thoughtfulness of details and eagerness in discussing the results have stimulated the investigation.

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THE REGULATION OF THE SUPPLY OF ENERGY IN MUSCULAR CONTRACTION.

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(*From the Physiological Laboratories, Cambridge and Manchester.*)

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In 1914 an investigation was carried out by one of us(1) of the heat produced in the prolonged isometric contraction of a frog's sartorius muscle. The observations were made at room temperature, viz. at about 15° C. At that time the preponderating effect of temperature was not realised, and no particular notice was taken of small changes of temperature: it was difficult moreover with the apparatus employed then to work with any great accuracy at any temperature other than that of the room: it seemed desirable therefore to confirm and amplify the 1913 results by the method described in our recent paper(2) which makes it possible to work with equal ease at all temperatures. This investigation has led (*a*) to a theory of the physico-chemical mechanism regulating the supply of energy in an excited muscle, and (*b*) to a general relation between the mechanical and thermal responses, for all durations of stimulus.

Method. The heat was determined by the employment of the nickel-gold thermopile referred to in our previous paper(2). A calibration experiment was carried out in each case, on the dead muscle, to enable the heat produced to be expressed in absolute measure. This method of calibration is entirely satisfactory, the galvanometer deflections being under all conditions directly proportional to the rate of heat-production by the warming current, and to the duration of the warming. There is no basis for Snyder's(7) suggestion that "electrical currents passing

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through the dead muscle may activate... oxidations in the disintegrating tissues," and so lead to errors. Such errors, if they existed, would not remain exactly proportional to the amount of electrical energy liberated in the muscle over long periods of time, and under all conditions. The whole of this investigation depends upon an accurate measurement of the quantities observed in absolute units of heat or work, and the method of calculating the sensitivity adopted by Snyder would in our case lead to a result 300 p.c. too large, and render the investigation worthless.

The heat H liberated in any portion of muscle of length l and of mass m has, for reasons given below, been expressed in terms either of H/l or of H/m, *i.e.* in terms of heat per centimetre length, or of heat per gram. Heat has usually been expressed in gram-centimetres instead of calories, partly because a gram-centimetre is a more convenient size of unit in dealing with muscles, and partly because by this means a direct comparison is possible between the thermal and the mechanical response. Gram-centimetres can be reduced to calories by dividing by $4{\cdot}24 \times 10^4$. The temperature of the muscle was varied by varying the temperature of the water inside the Dewar flask. The rise of tension T of the stimulated muscle was recorded on a moving surface, either (*a*) by an ordinary writing tension-lever, or (*b*) usually, in order to ensure greater quickness and accuracy of response, by photographic means (2. p. 116). In a number of experiments the tension in the longer contractions began to fall appreciably before the stimulus ceased: such experiments have been neglected.

The stimulus employed was always maximal, and produced either by a single induction shock, or by a single phase alternating current of 90 periods per second, giving therefore 180 stimuli per second. Its E.M.F. could be varied as required, and the heat produced by it determined if necessary: as a matter of fact the heating effect of the stimulating current was always negligible. The frequency of stimulation was not varied, since it was shown in a previous paper (1) that the frequency is without effect on the heat production, provided the tetanus is complete.

It should be noted that the "heat" referred to in this paper is that liberated in the initial three phases (2. p. 126) of the contraction, and includes none (or practically none) of that produced in the fourth recovery phase. It is read directly as the maximum deflection of the galvanometer, corrected if necessary in the longer contractions for the (small) loss of heat occurring before the maximum is reached. The recovery heat-production takes place too slowly to have any appreciable effect in the first few seconds involved.

The contractions have been isometric throughout. There is no particular virtue in the isometric contraction except that it eliminates one variable (the length of the muscle) and leaves the observer free to examine the elastic changes in the muscle uncomplicated by a change of length. The simple relation found below between the heat-production, the duration of the stimulus, and the mechanical response, could never have been established by a study of the more complicated case of a muscle allowed to shorten. The thermometric apparatus now available makes it possible to work on muscles allowed to shorten, without danger of disturbances produced by differences of temperatures along their length, and in a later paper we hope to deal with forms of contraction other than the isometric. Here we consider only the simplest case.

The method of conducting the experiment was as follows. As soon as the muscle had settled down to a constant temperature on the thermopile, usually in oxygen but sometimes in oxygenated Ringer's solution, it was given a few preliminary shocks and then a series of stimuli, increasing in duration from a single shock up to (say) two seconds, and diminishing again to a single shock. Each stimulus therefore was given twice, once in the increasing and once in the decreasing series, and an attempt made to eliminate the effects of fatigue by taking the mean of the two readings. After such a series, which was taken as representative of one temperature, the series was sometimes repeated at another temperature. It was often found that the results at a second temperature were unsatisfactory, owing to depreciation in the condition of the muscle due to the severe exercise, and such results have been neglected. Indeed it is probably best in general to trust to experiments made on fresh muscles, and to use a pair of muscles at one temperature only. In all the experiments given here, only the first series is given. By expressing the results in absolute units one experiment is made directly comparable with another. The method of eliminating the effects of fatigue by repeating the observations in the reverse order is very satisfactory, as is shown by the strikingly straight ends to the curves of Fig. 1. The results have been expressed in four ways:

a. By considering only H/m, the heat produced per gram of muscle in a maximal contraction, and paying no attention to the tension. The relation between H/m and x, the duration of the stimulus, is shown in Fig. 1, for five different temperatures. This relation will lead us to a hypothesis as to the manner in which the supply of energy is regulated in the muscle.

b. By considering H/Tl, the heat produced per centimetre length of muscle per gram weight of tension set up.

c. By considering H as a function of x and T. It will be shown that the heat liberated is the sum of two quantities, one being proportional to the duration of the stimulus, and the other to the potential energy set up.

d. By considering the "tension-time" set up. The "tension-time" is defined as the area of an isometric tension-time curve (3) and is of the dimensions of momentum (mass × velocity). It is equal to the momentum set up by the muscle in pulling horizontally at a large freely suspended mass. We shall show that a muscle excited isometrically by a prolonged stimulus liberates "tension-time" at a constant rate from the start: we shall then give the relation between the tension-time and the heat-production.

1. The relation between heat-production and duration of stimulus.

After a prolonged series of maximal stimuli at one temperature, followed by an interval of about an hour to allow the muscle to settle down to another temperature, the conditions of the muscle are apt to be lowered and less heat to be evolved from a given stimulus. It is

Table I. The relation between heat-production, duration of stimulus, and temperature. Many observations were made, at various times of year, but owing to the inadvisability of comparing observations made at one season with those made at another we have given here all the experiments made between May 22nd and August 10th and those only. Heat production given in gram-centimetres (not calories) per gram of muscle. Observations given so, [250], have been obtained by interpolation. The means at each temperature are plotted in Fig. 1.

Temp.		Duration of stimulus: seconds											
C.	Month	·00	·01	·02	·05	·10	·20	·40	·80	1·2	1·6	2·0	2·4
0°	May	[219]	215	224	255	288	343	445	610	720	835	950	1060
„	July	275	273	280	321	380	495	650	895	1120	1320	1500	1660
5°	May	[207]	214	256	309	366	486	690	995	1230	1470	1700	1930
„	July	215	[223]	265	315	381	518	740	1130	1410	1650	1900	[2110]
10°	May	[165]	210	264	382	495	715	990	1470	1880	2250	2610	—
„	July	192	[247]	310	495	700	1040	1560	2140	2700	3250	3750	—
„	Aug.	169	[196]	245	324	416	600	890	1270	1530	1810	2100	—
15°	June	192	219	283	430	610	880	1310	1980	2650	3330	[3950]	—
„	July	138	[155]	246	360	485	740	1085	1680	2240	2840	3430	—
„	„	152	200	337	520	798	1200	1670	2470	3200	3900	4680	—
20°	May	[122]	150	260	415	605	945	1400	2250	3170	4040	—	—
„	July	117	138	283	530	830	1270	1720	2610	3450	4300	—	—
„	„	123	159	300	555	805	1250	1850	2940	4000	5050	—	—
„	„	128	[154]	295	545	830	1220	1780	2700	3600	4600	—	—

inadvisable therefore to make observations at different temperatures on the same muscles. Our procedure has been to express the heat-production in calories (or gram-centimetres) per gram of muscle, and to assume that the average condition of a fresh muscle (in a large number

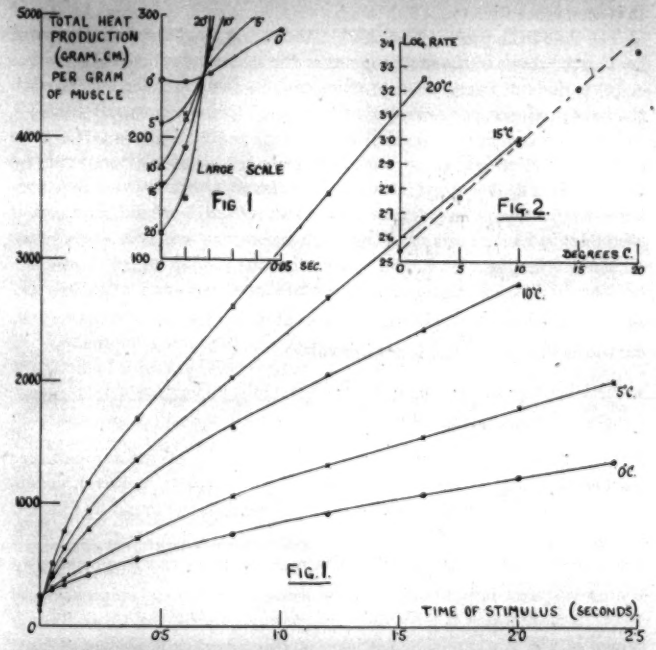

Fig. 1. Relation between heat-production H, duration of stimulus x, and temperature. The larger diagram shows the curves up to 2·0 seconds on a small scale; the upper diagram on the left shows the initial shape of the curves up to 0·05 second, on ten times the scale.

Fig. 2. Temperature coefficient of the rate of heat-production when a steady state has been reached. Horizontally temperature, vertically the logarithm of the heat-production per gram, per second duration of stimulus, as measured from the curves of Fig. 1.

of experiments) is constant for a given time of year. The results of a number of experiments are given in Table I, and taking the average of several experiments at each temperature they are shown graphically in Fig. 1. It should be noted that Fig. 1 does not give the relation between

heat-production and time, but between heat-production and duration of stimulus: the heat produced by a stimulus at any given moment does not appear *at* that moment (2. p. 111). We propose therefore to adopt the symbol x to denote duration of stimulus and t to denote time. It is seen from Fig. 1:

(1) that in a prolonged stimulus there is an extensive outburst of heat as the result of the first moment of the stimulus, and that successive equal periods of stimulation produce progressively smaller effects until the heat produced per second of stimulation attains a constant value;

(2) that the single maximal shock (represented by $x = 0$, *i.e.* zero duration of stimulus) gives considerably more heat at low temperatures than at high, while a longer stimulus gives far more heat at high temperatures than at low. The curves cross at about 0·017 second duration of stimulus: in other words a change of temperature has little or no effect on the energy liberated in a maximal stimulus lasting for 0·017 second.

The final steady value of the heat produced per gram of muscle per second of stimulus $\frac{1}{m}.(dH/dx)$, represented by the final slope of the curves in Fig. 1, has the following values:

Temp.	0° C.	5° C.	10° C.	15° C.	20° C.	25° C.	30° C.	35° C.	37° C.
gm. cm.	350	585	980	1640	2740	[4580]	[7650]	[12800]	[15700]
Calories	0·0083	0·0138	0·023	0·039	0·065	[0·108]	[0·18]	[0·30]	[0·37]

These values have been smoothed off by the aid of the temperature coefficient, found as shown below, and the numbers in square brackets extrapolated by its aid. The temperature coefficient is found as follows: the production of heat in the final steady state per gram of muscle per second of stimulus $\frac{1}{m}.(dH/dx)$ is read off from the final slopes of the curves, and it is found that the effect of a rise of temperature of 10° C. is to increase it 2·8 times over the range 0° C. to 15° C. This is shown in Fig. 2 where the logarithm of this quantity is plotted against temperature and shown to give a straight line. The rate therefore at which a stimulus liberates heat is governed by a temperature coefficient of the same size and exponential type as is an ordinary chemical reaction, and we may expect therefore that the regulation of the supply of energy to the muscle in a prolonged contraction is chemical in nature.

Various important questions arise in connection with these results:

(*a*) Why do the earlier shocks of a stimulus cause so much greater a liberation of energy than the later ones? (*b*) What is the nature of the final steady outflow of energy under a prolonged stimulus? (*c*) Why does an increase in the frequency of stimulation (1. p. 317) have no

effect on this steady outflow? (*d*) Why does a rise of temperature have so large an effect in increasing this steady outflow? (*e*) Why does a rise of temperature decrease the heat produced as the result of a single shock?

These questions go to the root of the problem of summation of contractions, and any theory of muscular activity must face them. We do not propose here to do more than indicate the lines on which we feel an explanation should proceed, the *type* of mechanism by which we believe the supply of energy is regulated in the muscle.

Imagine an elastic bag connected by a narrow pipe to a large reservoir of compressed air: the bag stretches and takes up air until the pressure in it is equal to that of the reservoir. A valve in the bag is now opened and the air rushes out, in large amount at first and then at a diminishing speed, until the rate of the outflow reaches a steady value determined by the size of the pipe connecting the bag to the reservoir. Similarly, interpreting this mechanical model in a chemical sense, let us suppose the muscle to possess a store (endowed with certain "elastic" properties to be described later) of the chemical bodies whose explosive liberation is the immediate cause of the mechanical response. This store is momentarily opened by a shock, *e.g.* by means of a momentary change in the permeability of its surface, and the chemical bodies [*e.g.* lactic acid] appear outside and cause the mechanical response. Following stimuli produce a similar though smaller outflow, as the elastic store becomes depleted. Simultaneously the store begins to be replenished along certain channels, not mechanical but chemical in nature, from the larger reservoir of available sugar or glycogen in the muscle. The mechanical model can be represented by the upper diagram of Fig. 3, while the hypothesis can be given a more concrete chemical form as in the lower diagram. Let us suppose that contraction is due to the appearance of a chemical

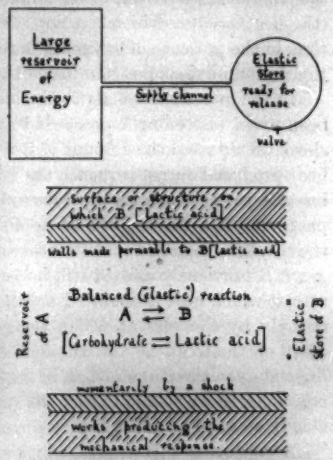
Large reservoir of Energy
Supply channel
Elastic fibers ready for release
valve
Surface or structure on which B [Lactic acid]
walls made permeable to B [Lactic acid]
Reservoir of A
Balanced (Elastic) reaction
A ⇄ B
[Carbohydrate ⇄ Lactic acid]
Elastic store of B
momentarily by a shock
works producing the mechanical response

Fig. 3. Diagram of mechanism regulating the supply of energy in the muscle. Upper figure mechanical model, lower figure proposed chemical system.

body B [lactic acid] in the neighbourhood of certain surfaces or colloidal networks of the muscle and that in relaxation B is removed from the seat of its action by some physical or chemical means (*e.g.* neutralisation). Let us suppose next that B is produced from some chemical body A, of which there is a large supply in the muscle; these hypotheses are not *ad hoc*, they are generalisations from previous work on the lactic acid and heat-production of muscle. In the system shown in Fig. 3, the large reservoir represents a large store of the body A: the supply channel is the chemical reaction (under the agency of a katalyst) $A \rightarrow B$ and the "elasticity" of the store of the body B is provided by the balanced nature of the reaction $A \rightleftarrows B$. We may suppose that the equilibrium exists inside some narrow space whose boundaries are impermeable to the active agent B [lactic acid]. A shock causes in these boundaries a momentary rise of permeability to B, this rise of permeability being manifested by the electric change; B immediately passes out: once outside it causes a rise of tension in the colloidal system there, and the mechanical response ensues. Relaxation is then produced by the neutralisation, removal, or combination of B, with an evolution of heat (the heat produced in relaxation, as we have shown elsewhere(2)) and then in the presence of oxygen B is removed by oxidation, or by restoration to its previous position inside the "reservoir," or by both(3).

In the meantime, owing to the loss of B, the equilibrium $A \rightleftarrows B$ has been upset and more A proceeds to be transformed into it. If now the shock be repeated the amount of B which passes out, and the amount of heat produced in consequence are less, because the concentration of B has not yet attained its previous value, and there is less of it ready to pass out. If a third shock be applied still less of B passes out, and if the shocks be repeated at short intervals the space in which the equilibrium $A \rightleftarrows B$ previously existed will be kept depleted of B: consequently the rate at which heat is liberated and B appears outside will be determined entirely by the rate at which the reaction $A \rightarrow B$ can go on, and not at all on the frequency of stimulation. The velocity of this reaction will depend upon the temperature, in the same way as that of other chemical reactions, so we should expect the rate of production of heat during the steady state to be affected by temperature as in the manner observed.

According to this scheme, which in detail is capable of wide modification, the heat is produced by any or all of the three causes: (*a*) by the breakdown $A \rightarrow B$; (*b*) by the action of B on the colloidal system outside; and (*c*) by the neutralisation, removal, or combination of B in relaxation. Each of these factors would give us an amount of heat proportional to

the extent of the breakdown A → B, so that the production of B from A should be proportional to the liberation of heat.

The "elastic" property with which it is essential to endow the system to explain the sudden outburst of energy at the beginning of excitation is a necessary consequence of the balanced nature of the reaction. So long as B is at a low "pressure" (*i.e.* concentration) the reaction goes →, diminishing in speed as the pressure of B increases. Finally a considerable amount of B is formed, and equilibrium is attained, the store of B being available for immediate explosive release on the application of a stimulus.

The hypothesis sketched above (*a*) is in general agreement with what is already known as to the chemical and physical accompaniments of muscular activity; (*b*) explains the greater effect of the earlier stimuli, the final steady state attained, and the independence of frequency of excitation; (*c*) explains the large effect of temperature on the rate of heat-production during the steady state; and (*d*) explains the effect of a fall of temperature in increasing the heat-production following a single maximal shock: *the change of permeability caused by the single shock lasts longer at the lower temperature* (as does the electric response) *and therefore allows more* B *to escape*, and *so more heat to appear*.

We believe that this general hypothesis will be found to give a consistent picture of the facts. We have purposely avoided making it too definite, as this can be done only after more specific chemical investigations have been made. Considerations of energy can never enable one to describe the precise mechanism but only the general sequence of events. With the aid of subsidiary hypothesis however it is possible to fit a large number of ascertained facts into the scheme: *e.g.* the onset of chloroform rigor may be supposed to be due to a permanent change in the permeability of the walls caused, *e.g.*, by the solvent effect of the chloroform; or the steady resting liberation of energy of a muscle to a small resting permeability of the walls: or the fact that less energy is liberated when the muscle is allowed to shorten to the fact that the area of the walls is smaller, and less energy can escape: or the prolonged contraction and evolution of heat induced by a shock after treatment with veratrine to a great increase in the time during which the permeability of the walls enclosing the store of B (lactic acid) is maintained. The reader can easily supply further examples for himself.

The absolute value of the rate of liberation of energy is of general, rather than of specific interest. The quantities given above represent, at various temperatures, the maximum sustained rate of output of energy

of which the muscle is capable. The observations have not been continued beyond 20°, owing to the rapid depreciation of a frog's muscle kept at higher temperatures. If however we extrapolate to 37° by means of the temperature coefficient we find that at 37° (if it did not go into rigor) the frog's sartorius muscle could attain a maximum sustained rate of output of energy of 0·37 calorie per gram of muscle per second. This does not include any heat produced in oxidative recovery processes, but only that liberated in the initial breakdown. Including the recovery processes the sustained rate would be about 0·70 calorie per gram per second.

2. The heat-production in relation to the tension developed and maintained.

This was investigated previously at 15° C. (1). The heat H was determined as before in any length l of muscle (either the whole length or the length between the electrodes) and divided by T the maximum tension set up. It is clear that H must be divided by l, otherwise H/T has no absolute meaning, since by increasing the length of the muscle —but not the cross-section—we can increase H without increasing T. A change in cross-section however affects both H and T equally in a maximal contraction, so need not be considered. Moreover if T be measured in grams wt. and l in cm., Tl is obtained in gram cm., so that if H be expressed (as has been done here) in gram cm. H/Tl becomes a *number* of no dimensions.

The values of H/Tl were observed on fresh muscles for stimuli increasing in duration from that of a single shock up to 2·0 seconds, and decreasing again to a single shock, and the mean was taken of the two values of H/Tl for each duration. If a second series be taken the values of H/Tl may differ appreciably from those recorded in the first series: in other words, employing the term "efficiency" in the loose sense of

$$\frac{\text{(tension maintained)}}{\text{(energy supplied in maintaining it)}}$$

the "efficiency" of the muscle has changed as the result of exercise. It is inadvisable therefore to perform more than one series of experiments on the same muscle, so that experiments at different temperatures must be made on different muscles. Hence to avoid errors due to variations in individual muscles it is necessary (*a*) to compare results obtained at one time of year only, and (*b*) to make a large number of experiments. The results of our experiments are collected in groups in Table II. It is seen that the "efficiency" of the muscle in developing and maintaining

a prolonged tension is less in July than in May or November. The bearing of this fact is discussed below, but for the moment it is clearly better to avoid taking the means of numbers differing as widely as those in

TABLE II. Relation between H/Tl and x for various constant temperatures, where H is total heat produced in length l of muscle, T is the maximum tension developed, and x is the duration of the stimulus in seconds. H and Tl are both expressed in gram cm. so that the quantities given in the Table are simple numbers.

Temp. C.	Month	Duration of stimulus: seconds										
		·00	·01	·02	·05	·1	·2	·4	·8	1·2	1·6	2·0
0°	May	—	·190	·200	·207	·220	·241	·287	·381	·462	·550	·609
,,	,,	—	—	·190	·196	·209	·230	·277	·353	·425	·495	·572
,,	,,	—	184	·191	·197	·205	·221	·270	·368	·436	·510	·558
,,	,,	—	·180	·182	·185	·201	·218	·264	·345	·410	·484	·550
,,	July	·296	·300	·304	·305	·330	·370	·425	·530	·626	·708	·790
,,	Aug.	·320	—	·325	·345	·362	·368	·416	·495	·570	·615	·695
,,	Nov.	·240	·242	·244	·260	·280	·302	·360	·440	·520	·580	·640
5°	May	—	·177	·190	·207	·225	·272	·345	·471	·570	·685	·748
,,	,,	—	·201	·211	·221	·240	·285	·365	·525	·655	·760	·890
,,	,,	—	·170	·188	·207	·228	·270	·347	·479	·585	·696	·810
,,	July	·252	—	·270	·287	·305	·350	·424	·590	·745	·858	·985
,,	Aug.	·260	—	·263	·274	·271	·320	·386	·510	·602	·725	·795
,,	Nov.	·260	·262	·265	·285	·310	·360	·440	·580	·680	·765	·850
10°	May	—	·198	·215	·243	·269	·330	·445	·611	·780	·925	1·100
,,	,,	—	·209	·213	·229	·238	·282	·384	·565	·706	·840	·981
,,	,,	—	·183	·196	·220	·255	·322	·410	·593	·760	·890	1·030
,,	July	·310	—	·330	·367	·410	·502	·588	·860	1·110	1·340	1·570
,,	Aug.	·236	—	·261	·295	·325	·378	·490	·645	·845	1·000	1·170
,,	Nov.	·280	·284	·290	·305	·340	·410	·540	·775	1·020	1·260	1·470
15°	May	—	—	—	·244	·275	·342	·468	·704	·930	1·120	1·320
,,	,,	—	—	—	·224	·251	·309	·425	·640	·841	1·045	1·230
,,	,,	—	·169	·179	·213	·246	·312	·435	·649	·871	1·062	1·295
,,	July	·260	—	·304	·354	·390	·476	·655	1·010	1·340	1·670	1·980
,,	,,	·265	·265	·320	·369	·438	·542	·700	1·050	1·360	1·670	2·000
,,	Dec.	·210	·250	·275	·330	·380	·460	·600	·870	1·140	1·400	1·660
20°	May	—	[·181]	·201	·225	·280	·381	·598	·940	1·290	1·570	1·910
,,	,,	—	·166	·177	·210	·240	·337	·481	·756	1·035	1·340	1·650
,,	,,	—	·170	·195	·239	·292	·390	·579	·924	1·230	1·580	1·900
,,	July	·226	·241	·302	·387	·460	·628	·885	1·440	1·940	2·300	—

Table II, and we have averaged the May results only in Fig. 4. In that figure the values of H/Tl are plotted against x, the duration of the stimulus, for various constant temperatures. From the figure we see:

(*a*) that H/Tl for a shock or a very short stimulus, is practically unaffected by a change of temperature, the curves for different temperatures all starting at about the same point;

(*b*) that the curve relating H/Tl to x the duration of the stimulus rapidly becomes linear, *the rate of heat-production* becoming proportional to the tension maintained;

(c) that a rise of temperature increases and a fall of temperature diminishes the value of H/Tl for a given x, the "efficiency" of the muscle in maintaining a force being much decreased by a rise of temperature.

With regard to (a), the fact that the value of H/Tl observed in the case of a single shock is independent of temperature, contradicts an observation made by one of us ten years ago with much less efficient

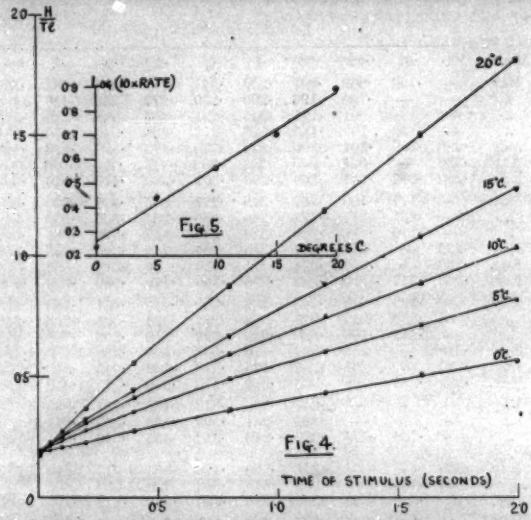

Fig. 4. Relation between H/Tl and duration of stimulus, for different temperatures. The curves all start from the same point, corresponding to H = Tl/5·5, and rapidly become linear.

Fig. 5. Temperature coefficient of rate of heat-production per cm. of muscle per gram weight of tension maintained. Horizontally temperature, vertically logarithm of the above quantity. Note the linear relation which corresponds to an increase of 2·0 times for a rise of 10° C.

methods (9), though as a matter of fact it adds another argument to the general conclusions of that paper that the tension bears a constant relation to the heat-production for a wide variation of the external conditions. The erroneous nature of the conclusion drawn then, that H/T diminishes as the temperature is raised, was probably due to a tension-lever, with too much inertia, "overshooting" in the more rapid

twitches at a higher temperature, and to the relatively large effects of fatigue in the case of a muscle raised to such temperatures as 26° or 28° C. Whatever be the explanation however of the previous erroneous result there can be no doubt that in fact H/Tl in a single isometric twitch is very nearly, if not quite, independent of temperature[1]. If the maximum tension set up be proportional to the total quantity of the body B [lactic acid] allowed to escape as the result of a stimulus, we might expect the tension to be proportional to the heat-production. In any case the observed constancy of H/Tl is further evidence that the tension developed in an isometric contraction is the most fundamental expression of the mechanical response.

With regard to (*b*), the observation that H/Tl rapidly becomes a linear function of the duration of stimulus confirms the results of two previous papers (9. p. 30 and 1. p. 320) over the whole range of temperatures investigated. It seems probable that a prolonged stimulus sets up a steady stream of the body B which acts upon the sensitive protein substances outside and is "removed" in the processes of relaxation at a steady rate, the tension at any moment being proportional to the amount still present. When a steady state is attained, as much B is liberated per second as is removed, and a steady concentration is maintained in the "active" spaces of the muscle so long as the supply and the removal are not interfered with. Thus the tension maintained should become proportional to the *rate* of heat-production.

With regard to (*c*) we may express the effect of temperature more exactly, as we did above in the case of the heat-production. The steady slopes attained by the curves of Fig. 4 were measured up and their logarithms taken: the numbers obtained, which are the logarithms of the rate of increase of H/Tl per second of stimulus, are plotted against temperature in Fig. 5. The result is a straight line, showing that the rate of increase of H/Tl per second of stimulus has a temperature coefficient of the same exponential type as an ordinary chemical reaction, increasing 2·0 times for a rise of 10° C. A similar calculation from the

[1] Prof. Meyerhof of Kiel (who saw the MS. of this paper) expressed privately to one of us a belief that this conclusion might, on further investigation, prove inconsistent with the facts. Since this paper was written we have therefore investigated the question again in a different manner, making readings of H/Tl on the *same* muscle at a succession of different temperatures. It is possible to do this if only a few shocks be given at each temperature, for then the muscle may maintain its condition for long periods. The experiments themselves will be described in a later paper, but it may be stated here that they are entirely consistent with the conclusion that H/Tl in a twitch is independent of temperature.

July results of Table II gives a temperature coefficient of about 2·6, and from the November results a temperature coefficient of about 2·4. We may take the mean temperature coefficient as being about 2·3. Before discussing the meaning of this high temperature coefficient we should note that the rate of increase of H/Tl per second of stimulus is of the dimensions minus one in time, and represents a *rate of proportional change, e.g.* it is of the same order of dimensions as the velocity constant k of the chemical reaction, $X \rightarrow Y$ in which $d[Y]/dt = k[X]$. Thus the rate of increase of H/Tl is a quantity which, unlike some quantities, we can reasonably endow with a temperature coefficient of the usual type and the fact that it is so high suggests that the velocity of some chemical reaction is the basis of it. Now if a constant stream of the chemical body B [lactic acid] is passing out, accompanied by a constant production of heat, its effect at any given spot—which depends upon its concentration there at the moment—is determined not only by the rate at which it is being supplied but by the rate at which it is being removed. The rate of removal will presumably be governed by some equation of the type (rate of removal) = k (concentration still present), while when the steady state is attained we must necessarily have (rate of removal) = (rate of supply). Thus during the steady state we should expect to find

(rate of supply of B) = k (concentration of B)

or since the rate of supply of B is measured by the rate of production of heat, and the concentration of B by the tension set up,

(rate of production of heat) = k (tension set up).

This agrees with the observed facts. Now suppose for the moment that the constant k is practically unaffected by the temperature, or in other words that the removal of B [lactic acid] is of a simple physical nature (*e.g.* diffusion). Then our last equation would show that in the final steady state the rate of production of heat divided by the tension set up should also be practically unaffected by the temperature. Our experiments show that this is not the case, and we must conclude rather that the constant k is largely affected by the temperature, or in other words that the removal of B is effected by chemical means. Thus *the high temperature coefficient of the rate of increase H/Tl with duration of stimulus points to the fact that the process of relaxation is due to removal of the active agent in contraction from the seat of its action by some chemical means.* This last deduction can be made in another way which is a striking confirmation of the general validity of the hypothesis sketched above. It is well known that the course of the mechanical response is consider-

ably affected by temperature, and we have shown (2. p. 117) that the "rate of relaxation" is increased about 2·2 times by a rise of 10° C. Thus the constant k defining the rate of removal of the active agent in the contraction might be expected to increase about 2·2 times for a rise of 10° C. and this agrees well with the mean value 2·3 of the temperature coefficient actually found for H/Tl. We may conclude therefore that the higher "efficiency" of the muscle in maintaining a force at a lower temperature is due to the fact that the fundamental processes accompanying relaxation go on much more slowly at a lower temperature, being in fact chemical processes of some kind with a high temperature coefficient.

The absolute value of the steady heat-production, per second of stimulus, per centimetre length of muscle, per gram weight of tension maintained, was given in the previous paper(1) as being 15×10^{-6} calories at 15° C. The values for 15° C. deduced from the above Tables have an average for May, July and November, of about 14×10^{-6} calories, which is in satisfactory agreement with the previous value obtained with very different apparatus. It is possible now to state the value of this quantity at all temperatures, as is done in Table III.

TABLE III. Absolute values of heat-production per cm. length of muscle, per gram weight of tension maintained, per second stimulus, in a prolonged contraction (d/dx H/Tl). Mean values taken for May, July and November, for sartorius of *Rana temp.* Mean temperatures coefficient found = 2·3 for 10° C. The values above 20° C. were obtained by extrapolation with the aid of the observed temperature coefficient.

Temp.	0° C.	5° C.	10° C.	15° C.	20° C.	25° C.	30° C.	35° C.	37° C.
Calories $\times 10^{-6}$	4·1	6·2	9·4	14	22	[33]	[50]	[75]	[87]
Gram cm.	0·17	0·26	0·40	0·61	0·92	[1·4]	[2·1]	[3·2]	[3·7]

According to previous work(4) the potential energy of an excited muscle is about Tl/6, and this has been confirmed by recent work of Meyerhof's(5) and by the results given in the next part of this paper: hence in order to *maintain* a state of potential energy E in a muscle, the following amounts of energy have to be supplied per second.

Temp.	0° C.	5° C.	10° C.	15° C.	20° C.	25° C.	30° C.	35° C.	37° C.
Energy supplied per second = E ×	1·0	1·6	2·4	3·7	5·5	[8·4]	[13]	[19]	[22]

This shows the extraordinary inefficiency of the prolonged contraction of striated muscle at higher temperatures. It should be noted that the calculation takes no account of the energy wasted in recovery processes, so that taking the complete process into account the numbers given above should be about doubled. It would be interesting to compare

these numbers with those deduced from the oxygen consumption of a man holding a heavy weight.

Since the relative economy with which a force is maintained by a muscle is, physiologically speaking, a very important quantity, it is desirable to have a term to denote it. The amount of heat liberated per second of stimulus, in maintaining a force T in a length l, viz. (dH/dx)/Tl, the quantity given in the last row of Table III is clearly a measure of the wastefulness of the muscle in the process, and we may denote its reciprocal (Tl)/(dH/dx) by the term "*the efficiency of a muscle in maintaining a constant force.*" The efficiency of the muscle in maintaining a constant force is decreased very largely by a rise of temperature, to the extent of 2·3 times (on the average) for a rise of 10° C. It is affected also by a variety of other factors. For example, the immediate result of exercise is to increase the efficiency of the muscle in maintaining a constant force, and to diminish the speed of response, while the July frog (which is in better condition and "training" and exhibits a more rapid twitch than the May or November one) shows a smaller efficiency. We may generalise by saying that any factor which slows the chemical processes accompanying relaxation will increase the efficiency of the muscle in maintaining a force. This generalisation has obvious physiological applications. Some muscles, *e.g.* unstriated muscles, relax exceedingly slowly, and these are the muscles occupied chiefly in maintaining a force for a long time: their high efficiency in so doing is a direct consequence of the slowness of their relaxation processes. Supposing the single twitch of all muscles to be produced with the same high efficiency, it is obvious that the muscle whose twitch takes 1000 times as long to run its course will maintain a force over a long period with 1000 times the efficiency. Or again, it is well known to athletes that heavy and exhausting exercise is very bad training for sports, such as sprint-running or jumping, where great quickness is required: the training which leads to quickness of contraction and relaxation is directly opposed to that which leads to economy, and immunity from fatigue, in slow, prolonged and heavy movements.

3. The heat-production in relation to the potential energy developed and the duration of the stimulus.

It was shown in Part 1 that H, the total initial heat-production, becomes a linear function of x, the duration of the stimulus, as x is increased. In Part 2 it was shown that H/Tl similarly becomes a linear function of x. It should be noted however that H, or H/Tl, does not

immediately become a linear function of x, and the divergence from the linear relation is considerable at the start, as is seen in Figs. 1 and 4. It is clear that H is made up of two factors, one of which is proportional to the duration of the stimulus. The question then arises, what is the second factor? Now a muscle has two functions, dynamical and statical, viz. (*a*) the production of potential energy to enable the animal to do work if required, and (*b*) the maintenance of a force to enable the animal to carry loads, to support itself, or to preserve its posture. These functions of a muscle are independent ones, and we might expect them to correspond to two different factors in the heat-production. If this be so, we should hope to find that H = (some function of the duration of the stimulus) + (some function of the potential energy developed). Now, as the stimulus is continued, the potential energy reaches a maximum and remains constant, while the heat-production H becomes a linear function of x the duration of the stimulus. Hence we might expect to find:

$$H = bx + (\text{some function of the potential energy E})$$

where b is some constant, depending on the temperature. In a previous paper (4. p. 461) it was shown that the potential energy developed in a muscle on excitation, as measured by the quantity Tl/6, is a fairly large fraction of the initial heat-production, amounting for a stimulus of 0·075 seconds, and under a moderate initial tension, to about 75 p.c. of the heat-production. This result has been confirmed by Meyerhof (5. p. 278) who finds, employing a different method and a different muscle, that on the average the potential energy of an excited muscle is about 80 p.c. of the total initial heat-production, being anywhere between 75 and 100 p.c. A further confirmation of the same fact is given by the experiments recorded in Table II and Fig. 4, where it is seen that for very short durations of stimulus the quantity H/Tl is the same for all temperatures and has, for the May results, the value (about) 0·18. Thus for the May results Tl/6 is about 93 p.c. of the total initial heat-production, at all temperatures. The previous value (75 p.c.) was also obtained in May and was for a stimulus lasting 0·075 second; reference to the 15° C. curve in Fig. 4 shows that a stimulus of 0·075 second gives a value of H/Tl of 0·23, corresponding to a value of Tl/6 equal to 73 p.c. of the heat-production. The agreement is very good, and it is obvious now that to ensure the highest efficiency the shortest possible stimulus should be employed, so that for a single shock and employing the English frog in May we may say that 93 p.c. is the value

of the efficiency as measured by the quantity Tl/6H. Practically therefore the initial heat-production is equal to the potential energy developed, or in other words practically the whole of the energy liberated by a muscle in the initial stages (*i.e.* apart from "recovery") of a twitch passes through the phase of potential energy before it appears finally as heat[1]. Returning to our original argument therefore we should expect to find, $H = bx + E$, or in other words the heat-production to be the sum of two factors, one equal to the potential energy developed, and the other to the duration of the stimulus multiplied by some appropriate constant. We have tested this deduction in the following manner, and so obtained a striking confirmation of the theory.

In all the experiments made in May the heat-production per gram of muscle has been plotted against x the duration of the stimulus, as in Fig. 1, and the final steady slope of the curve read off. H/m finally becomes equal, for large values of x, to bx + (some constant). Now b having been determined, the quantity (H/m − bx) can be calculated for all durations of stimulus from the observed values of H and x, and the mean values of (H/m − bx) for each temperature are given in Table IV. In the same Table are given the mean values of Tl/m. It is obvious at once that at all temperatures (H/m − bx) and Tl/m increase together and reach a maximum at or near the same duration of stimulus. The two quantities are plotted against one another in Fig. 6, when it is seen:

(*a*) that up to a certain limit of Tl/m, *i.e.* up to a certain duration of stimulus, (H/m − bx) is directly proportional to Tl/m;

(*b*) that beyond this duration, *i.e.* for larger values of Tl/m, the curve bends upwards, the ratio of (H/m − bx) to Tl/m increasing.

The same facts are shown by the results of July, Aug., Nov. and Dec.

Over the fairly wide range where (H/m − bx) is proportional to Tl/m, the constant of the proportion, viz. $k = \frac{H/m - bx}{Tl/m}$, is practically the same for all temperatures, and has the value 1/5·5. The fact that k is independent of temperature suggests that it has some real physical significance, while the agreement found between this quantity and the constant, 1/6, in Tl/6, the expression for the potential energy, is striking evidence that, at any rate for the shorter durations of stimulus, the quantity (H/m − bx) really does represent the potential energy developed

[1] It will be seen that the values of H/Tl given in Table II for months other than May (July, Aug., Nov. and Dec.) are considerably higher, averaging (for zero duration of stimulus) 0·26. This lower value corresponds to Tl/6 = 0·64 H. It may be that at other times of year the "efficiency" is really lower; or it may be that the potential energy is really greater than Tl/6.

in the muscle. The expression Tl/6 for the potential energy of a stimulated muscle was only intended to apply to the case of a single shock or a very short stimulus, and seeing that (H/m − bx) is equal, or approximately equal, to the potential energy of the muscle, so expressed, in the shorter stimuli, it is natural to assume that in the longer stimuli

TABLE IV. H/m is the heat-production per gram of muscle, b is the final steady rate of heat-production in a prolonged stimulus, x the duration of stimulus, T the maximum tension developed, l the length of the muscle, and m its mass. The means given are the results of 13 experiments on fresh muscles made in May.

		Duration Secs.							
		·01	·02	·05	·10	·20	·40	·80	1·20
0° C.	H/m − bx	213	221	236	254	281	317	355	359
b = 307	Tl/m	1170	1200	1290	1370	1490	1590	1660	1680
5° C.	H/m − bx	199	236	272	304	368	446	520	523
b = 525	Tl/m	1130	1260	1400	1550	1730	1860	1920	1930
10° C.	H/m − bx	197	234	286	324	399	491	555	—
b = 733	Tl/m	1035	1180	1400	1580	1730	1870	1910	—
15° C.	H/m − bx	193	280	356	413	502	580	—	—
b = 1450	Tl/m	1210	1710	1950	2160	2380	2400	—	—
20° C.	H/m − bx	160	235	311	380	484	497	490	—
b = 1910	Tl/m	1020	1410	1750	2050	2230	2280	2310	—

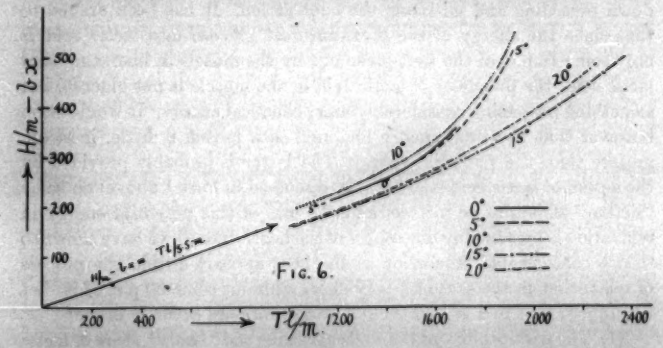

Fig. 6. Relation between (H/m − bx) and Tl/m. For description of symbols and discussion see text.

also the true measure of the potential energy set up is (H/m − bx). It is probable that in the longer stimuli the actual work which could be obtained from a muscle is less than (H/m − bx), because the viscous flow of the muscle under a prolonged tension may have degraded a considerable part of the potential energy into heat (10); nevertheless we may regard (H/m − bx) as representing either the actual potential energy

(as in the shorter stimuli), or as energy which has passed through the phase of mechanical potential energy before being degraded into heat by the viscous flow of the muscle under a prolonged contraction.

We may conclude therefore that the two separate functions of a muscle, the production of work, and the maintenance of a force, are reflected in two separate factors in the heat production. The total energy set free by a muscle in an isometric contraction is the sum of two separate quantities, (1) the energy appearing in, or passing through the phase of potential energy; and (2) an amount of energy proportional to the duration of the stimulus; and may be expressed in an equation,

$$H = bx + E$$

where H is the heat set free, x is the duration of the stimulus, b is a constant characteristic of the muscle, its temperature, etc., and E is the potential energy produced in it. In a short stimulus the energy appears almost entirely in the potential energy term, as mechanical energy ready to do work if required. In a long stimulus the energy is used almost entirely in maintaining the contraction.

This result bears upon our views of the nature of the chemical breakdown preceding and initiating the contraction. It has been argued (11) that since the energy of the breakdown of glucose into lactic acid is only some 16 p.c. of the heat given out by the muscle in liberating that lactic acid, the precursor of lactic acid in the muscle is not glucose, but something possessing considerably more chemical energy. It would seem however that the total energy liberated in a twitch is little, if at all, greater than the potential energy. The latter is probably provided by the action of lactic acid (the body B, discussed in Part 1 above) on some "active" structure or surface, and the loss of this potential energy in relaxation is presumably one source of the heat. Indeed we have shown (2) that a considerable proportion of the heat appears during the process of relaxation, in the case of a very short stimulus some 30 p.c. of it. Let us suppose that in a twitch the following processes occur in order:

(1) The permeability of the walls enclosing the "ready" store of lactic acid is increased by the shock, the lactic acid passes out, acts upon the "sensitive structure" of the muscle, liberating potential energy E and heat H_1.

(2) Carbohydrate breaks down to replace the lactic acid lost, with production of heat H_2.

(3) Relaxation sets in, the lactic acid is removed from the sensitive structure by some anaerobic chemical or physico-chemical process unknown, with production of heat H_3.

(4) Finally in the presence of oxygen the lactic acid is replaced in its previous position as carbohydrate (Meyerhof(5),(6)), about $\frac{1}{3}$ or $\frac{1}{4}$ of it however being lost in the process in supplying energy to "restore" the chemical condition of the muscle, and in giving out the "recovery heat-production"(12). According to this scheme the total heat given out in the initial as distinguished from the recovery phases is:

$$H = H_1 + H_2 + H_3$$

which is approximately equal to the potential energy E. The heat H_1 is liberated during the development of the mechanical response, the heat H_3 during its disappearance; while H_2, the heat derived from the reaction, carbohydrate → lactic acid, is liberated at some time unknown. If we adopt this scheme of the events happening in muscle we are relieved from the necessity of finding a lactic acid precursor other than carbohydrate, a chemical problem often and vainly attempted. We are reduced rather to finding a non-oxidative chemical or physico-chemical reaction capable of seizing on and "removing" lactic acid from the sensitive structures or surfaces of the muscle, and then of delivering up the lactic acid it has seized upon to the later and gradual processes of oxidative recovery. If we suppose that the bodies capable of combining with, and so of removing, the lactic acid, are limited in amount we can explain the existence of the "lactic acid maximum" as being due to the cessation of the reaction, carbohydrate → lactic acid, as soon as the products of the reaction begin to appear in a free form: and the slower relaxation of a muscle subjected to fatigue or the action of acid would then be due to the partial saturation of the bodies whose function it is to seize upon the lactic acid and so to reverse the process of contraction.

This view agrees largely with that of Meyerhof(14) who says: "Lactic acid is produced explosively at the 'verkürzungsorten': this releases a process possessing considerable free energy, whereby the elastic condition of the smallest fibrils is changed: the lactic acid is then removed by the attraction of the surrounding muscle-plasma, with an affinity at least as great as that of its original attraction to the fibrils." Meyerhof also looks for the energy liberated in contraction not in the energy of a built up precursor, but in that set free in the actions whereby lactic acid is attracted to and removed from the "sensitive" protein bodies of the muscle.

4. The tension-time production of muscle.

It was shown above (Part 2) that once a steady state has been reached the total heat produced in any interval is proportional to the

force maintained multiplied by the time during which it is maintained. The hope of finding some simple expression for the mechanical response, proportional to the heat-production from the beginning of the stimulus and not merely after a steady state has been attained, led us to examine the properties of the "tension-time," a quantity which is defined as the area of the isometric tension-time curve, or mathematically as $\int T dt$ where T is the tension developed and t the time. As a matter of fact, as is shown in Fig. 8, the "tension-time" is not exactly proportional to the heat-production; indeed the results of Part 3 would not be true if it were. The experimental investigation however of the properties of the tension-time has led to a striking simple relation.

It might at first appear that the tension-time is an artificial conception; as a matter of fact it is anything but artificial, and represents an important mechanical property of the muscle. Suppose that we have a large mass hanging at rest on a long vertical string; let us attach to it an inextensible horizontal string connected to a muscle, and stimulate the muscle. The momentum (mass × velocity) produced in the mass is equal to the tension-time produced by the muscle, and the faculty of a muscle for producing motion in heavy masses is determined therefore by its faculty for producing what we have here called "tension-time." This quantity can be determined experimentally in various ways, *e.g.* by allowing the muscle to accelerate a heavy mass, and measuring the momentum set up; the most accurate method however is the indirect one of making it record an isometric tension curve on a uniformly moving surface, and measuring the area of this curve. This method we have adopted throughout the present investigation, using either mechanical or photographic recording of the tension curves.

If we apply to the muscle a series of maximal stimuli starting from a single shock, increasing in duration up to two or three seconds, and returning down the series again to the single shock, we obtain a set of isometric tension-time curves whose areas can be measured up and recorded just as the heat-production in the experiments described above. There is no need here to give such a series of isometric curves, as they are similar to those recorded on p. 116 of our previous paper (2). If now the means of the two observations at any one duration be taken, in order to eliminate fatigue, we get the series of results which are recorded in Table V and shown graphically in Fig. 7. In order to make the results with different muscles comparable with one another it is necessary that the quantity determined as above should be independent of the dimensions of the muscle, and this we have secured by employing instead of the tension T the quantity Tl/m where l is the length and m the mass

of any portion of the muscle, this quantity being proportional to the tension developed per sq. cm. of cross-section of the muscle. The numbers in Table V and the curves in Fig. 7 have been given only up to 0·54 second duration of stimulus; in every experiment carried out (including

TABLE V. Tension-time production against duration of stimulus, for different temperatures. The tension has been expressed in terms of Tl/m (which is proportional to the tension per sq. cm. of cross-section), in order to make the results with different muscles comparable. [T is in grams-weight, l in cm., m in grams, and time in seconds.]

			Duration of stimulus								
Temp.	Month	Shock	0·015	0·030	0·045	0·075	0·12	0·18	0·27	0·39	0·54
0° C.	Oct.	418	409	425	500	525	598	695	815	945	1110
	Nov.	410	—	439	[512]	538	626	725	845	1009	1231
5° C.	Oct.	153	172	217	256	300	386	491	630	810	1100
	Nov.	139	175	193	231	295	387	513	670	870	1120
10° C.	Sept.	58	99	127	161	220	279	394	549	—	—
	Oct.	88	137	192	220	268	355	470	641	—	—
	Nov.	60	94	117	149	199	253	347	491	680	920
15° C.	Dec.	61	—	141	—	235	315	442	636	900	1230
	Dec.	61	—	153	—	246	340	481	693	980	1330

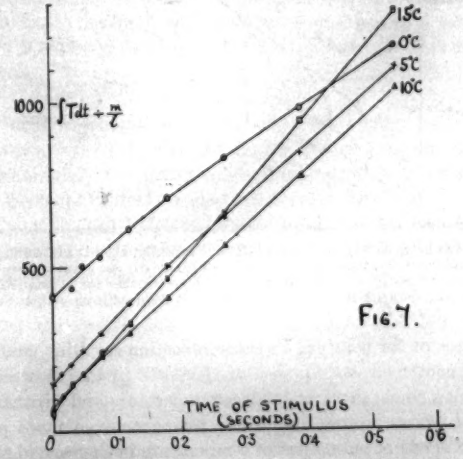

FIG. 7.

Fig. 7. Relation between "tension-time" (vertically) and duration of stimulus (horizontally). Note linearity of relation at all temperatures.

several with time of stimulus up to two or three seconds) the curves could not be distinguished from straight lines (especially at higher temperatures), and it seemed unnecessary to continue the curves further. Although the curve for each individual experiment approximates nearly

to a straight line there is not a very close agreement between the results of different experiments. This is due in part to the effect exercised by the condition of the muscle, but principally to the great effect of the initial tension, a factor the importance of which was not realised at the time these experiments were made(13). From Fig. 7 we see:

(*a*) that for a single shock the tension-time is very considerably increased by a fall of temperature; for long durations however the curves tend to cross, and more tension-time to be produced by the warmer muscle;

(*b*) that at all temperatures the relation between the tension-time and the duration of the stimulus is linear from the start.

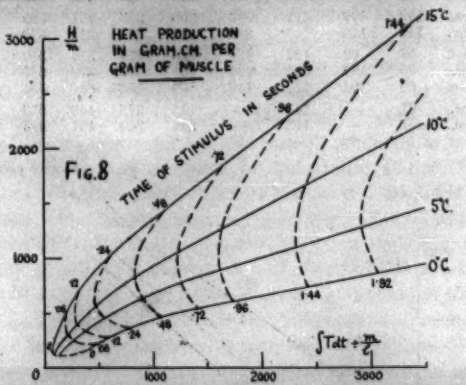

Fig. 8. Relation between heat and tension-time. Vertically, H/m in gram cm. per gram of muscle; horizontally, tension-time multiplied by length per gram of muscle. The full curves are for constant temperatures, the broken curves for constant duration of stimulus. The diagram shows the average results deduced from several experiments, made in Oct. and Dec.

The effect of temperature on the contraction resulting from a single shock is a combined consequence of the facts (i) that more energy is liberated by a single shock at a lower temperature and (ii) that a given quantity of energy causes, at a lower temperature, a more prolonged contraction. The opposite effect of temperature in the case of the longer stimulus is due to the fact that in this case far more energy is liberated at the higher temperature, and this more than compensates for the more rapid passing off of the effect of a given amount of it. The linearity of the relation shown in Fig. 7 is good, if empirical, evidence that the tension-time has some real physical (or physiological) significance in the economy of the muscle.

We will turn now to a short account of experiments in which simultaneous observations were made of the heat and of the tension-time produced by a muscle. The experiments were carried out in the usual manner, the heat H being measured in gram cm. of work per gram of muscle, and the tension-time in terms of $\int Tl\,dt/m$. The results are shown in Fig. 8, where we have plotted the two quantities concerned against one another (i) for constant temperature, and (ii) for constant duration of stimulus. For constant temperature and increasing duration of stimulus the relation between the heat and tension-time rapidly becomes linear, while for constant duration and increasing temperature the relation follows the curious broken curves shown in the figure. We are unable at present to draw any obvious deduction from these curves, but the striking linearity of the relation shown in Fig. 7 between tension-time and duration of stimulus suggests that the former has some real physiological significance, in which case its relation to the heat-production as given in Fig. 8 may be of value in some future investigation. In any case Fig. 8 shows in a convenient form the relation between the four quantities, heat, tension-time, duration of stimulus, and temperature, any two of these being determinable from the curves as soon as the other two are given.

Summary.

1. A single maximal shock produces considerably more heat at a lower temperature: a tetanic stimulus of about ·017 second duration produces practically the same heat at all temperatures; a longer stimulus produces far more heat at a higher temperature.

2. In a prolonged maximal contraction there is a sudden outburst of heat for the first moment of the stimulus, succeeding elements of the stimulus producing less and less effect until a steady state is reached in which heat is produced at a constant rate. In this final steady state the rate of heat-production has a temperature coefficient of the exponential type, increasing 2·8 times for a rise of 10° C.

3. For a single shock H/Tl has the same value for all temperatures, thus confirming the view that the tension developed in an isometric twitch is the best measure of the mechanical response. [H is heat-production, T is tension developed, l is length of muscle.] H/Tl increases as the stimulus is continued, becoming finally a linear function of the duration of the stimulus; in other words the heat-production becomes proportional to the force maintained. In this final steady state the rate of heat-production per unit of force maintained is largely increased by a rise of temperature, having an exponential temperature coefficient of 2·3 per 10° C.

4. In a contraction the heat produced is the sum of two factors, corresponding to the two functions of a muscle of maintaining a force and of doing work. This may be expressed by the formula $H = bx + E$, where E is the measure of the potential energy produced, x is the duration of the stimulus and b a constant characteristic of the muscle and its condition. This is the most general relation known between the mechanical and the thermal response.

5. The tension-time (the area of an isometric tension-time curve) is a linear function of the duration of the stimulus, from the first moment of the stimulus. For short durations it is less, for long durations it is greater at a higher temperature (cf. Fig. 8).

6. The heat produced per gram of muscle, per second of stimulus, in the final steady state of a prolonged isometric contraction, is given (p. 138) in absolute units of heat for a variety of temperatures.

7. The heat produced per cm. length of muscle, per gram wt. of tension maintained, per second of stimulus, in the final steady state, is given (p. 147) in absolute units. The results show a very low "efficiency" of the striated muscle in *maintaining a constant force*, especially at higher temperatures. On the other hand in a single twitch the "efficiency" in *producing potential energy*, as expressed by $\frac{1}{3}Tl/H$, is very high, approximately to unity.

8. The "efficiency" of the muscle in maintaining a constant force is increased by a fall of temperature, or by any other factor tending to increase the duration of the twitch resulting from a single shock. It is lowered by any factor increasing the quickness of the muscular response. These facts explain the high efficiency of the unstriated muscle in maintaining a constant force.

9. A physico-chemical mechanism is suggested to account for the manner in which the supply of energy is regulated in the muscle.

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THE REGULATION OF THE REACTION OF THE BLOOD.

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THE experiments described in this paper deal with the question of the relative parts played by various modes of buffering in the blood in regulating its hydrogen ion concentration[1]. The hydrogen ion concentration has been determined by the indicator method described by Dale and myself(7). I have shown in an earlier paper(10) that the hydrogen electrode method gives a p.H which is 0·2 lower than that given by indicators and have asserted that the true p.H is that given by the latter method. Apart from this, as will be shown in this paper, there is no difference of any significance between the results of the two methods. The indicator method, however, has the advantage that determinations can be quickly and easily made.

Definitions. The expression *the reaction of blood* will be understood

[1] The article by D. D. Van Slyke on "The Carbon Dioxide Carriers of the Blood" appeared in *Physiological Reviews* (I. p. 141) while this paper was in the Press. In his able treatment of the subject Van Slyke arrives by another route at several of the conclusions of the present paper, and it is regretted that a fuller discussion of his publication is not now possible.

to mean the reaction of the plasma (or serum); Parsons(26) has shown experimentally that there is no difference between the reaction of blood and that of its plasma under identical conditions, and I can fully confirm this observation. Though it is a matter of undoubted importance, little is as yet known with regard to the relation between the reaction of the contents of the intact corpuscles and that of the plasma.

The term *alkali reserve* is used, as by Van Slyke, to mean the percentage volume of carbon dioxide at N.T.P. held in chemical combination by a fluid which is in equilibrium with carbon dioxide at alveolar tension (42–45 mm. in the present experiments). The term *fixed carbon dioxide* is often used instead of alkali reserve, when there is some doubt as to the exact mode of fixation.

By *carbon dioxide content* is understood the total percentage volume of the gas at N.T.P. held both in the dissolved and in the chemically fixed state, at any stated carbon dioxide tension.

The *carbon dioxide reaction curve* (or R-CO_2 curve) is the graph expressing the relation between the reaction (p.H) of the fluid (ordinates) and the carbon dioxide pressure in mm. of mercury (abscissæ).

Methods. Human blood, and blood of the goat and horse, was drawn by suction from a superficial vein into a syringe or flask: rabbit's blood was allowed to drip from a small incision in an ear vein into a suitable receptacle: the blood of other species was usually obtained from the anæsthetised animals by arterial cannulæ. Most of the experiments have been made with blood rendered incoagulable by the addition of 0·1 p.c. of its weight of solid carbonate-free potassium oxalate. In one or two cases, where freshly defibrinated blood has been employed, no striking difference has been observed. The addition of oxalate in small amount is without influence on the reaction of the blood at a given carbon dioxide pressure; above 1 p.c., however, the correction for salt error becomes appreciable, as the following experiment shows.

Exp. 1. Defibrinated cat's blood was exposed at 18·5° to a pressure of 9·1 mm. carbon dioxide; samples were then run direct into three of the small dialysers. To the first dialyser no oxalate was added: to the second 7 mg. (to make ·23 p.c. in blood) and to the third 35 mg. (=1·15 p.c.). The reaction of the dialysates were respectively 7·5, 7·5, and 7·42.

It has been shown by de Corral(9) that the electrometrically determined reaction of blood is also unaltered by oxalate, and Van Slyke and Cullen(33) similarly found that the amount of oxalate added was, within wide limits, without effect on the alkali reserve of the plasma drawn from blood. Peters, Barr and Rule(28) have also found that 0·5 p.c. of potassium oxalate scarcely alters the total CO_2 fixed by blood

at a given CO_2 pressure. The experiments of Haggard and Henderson(13) moreover indicate that oxalated blood is preferable because it is more stable than defibrinated blood.

Equilibration of blood and other fluids with gas mixtures of varying composition was effected by horizontal rotation in saturator vessels of the pattern used by Barcroft: owing to the relatively large volume of fluid which was often present it was not always easy to obtain any desired pressure of carbon dioxide at one saturation, and in such cases a repetition of the saturation with a fresh gas mixture of similar composition was necessary. The saturation was effected either at room temperature (which varied little from a mean of 20°) or at 38° C. The former has many advantages, not the least of which is that post mortem changes in the blood are less rapid at lower temperatures. After five minutes' exposure to the gas mixture, the tap of the saturator vessel was momentarily opened to the air (or other gas with which the CO_2 was diluted) in order to equalise the pressure within and without; this was repeated again after a further five minutes' rotation. After equilibrium had been reached (about 15 minutes) and five minutes after the last barometric pressure equalisation, a sample of the gas was quickly withdrawn from the saturator and analysed in the small pattern Haldane gas analysis apparatus. The sample of blood, or other fluid, was then at once drawn off from the saturator and used.

Determinations of the reaction of the blood were made by the dialysis method as described by Dale and Evans(7). As indicators I have used neutral red and phenol red. Neutral red, I think, gives less trustworthy results than phenol red when the p.H of the dialysate is higher than 7·8: and in general in those cases I draw conclusions only from the observations made with phenol red. In the determination of the reaction of clear aqueous solutions of colourless salts the dialysis can be omitted. Corrections for the salt error of the indicator were always made when enough salt was present to make such a correction significant. When equilibration is carried out at body temperature, it is immaterial whether the subsequent dialysis of the blood is performed at 38° or at room temperature, a fact which agrees with the observation of Joffe and Poulton(19) that the alkali reserve of the plasma of blood equilibrated at body temperature is unaltered if the blood is allowed to cool during centrifugation.

When plasma was to be separated from equilibrated blood this was always effected after discharge of the blood direct from the saturator beneath the surface of a layer of liquid paraffin 1 cm. in thickness in

the centrifuge tube, and without any contact of the blood with atmospheric air. After centrifuging, the layer of plasma was removed with a capillary pipette, contact with the air after removal from the corpuscles being of no consequence.

Alkali reserve and carbon dioxide content were determined in the usual way by the Van Slyke(32) apparatus and are given to the nearest unit in percentages; oxygen capacities were estimated by the Barcroft-Haldane method, with the 1914 pattern Barcroft apparatus. Some parallel determinations were also made by means of the Haldane hæmoglobinometer. Plasma chloride was estimated by the simple and rapid method due to Rappleye(29), which I found to work sufficiently well even with oxalated blood. Nitrogen estimations were made by the Kjeldahl method.

For the determination of the corpuscular volume, the following simple modification of the capillary tube method, which I acquired from Dr Harold King, was found to give excellent results. A glass capillary is drawn, and a length of it A–B filled with blood. This length is taken from a part of the drawn capillary in which the taper is in one direction. The end A is now sealed, the capillary broken in the middle, and the end B sealed: the two tubes are then spun at high speed in the centrifuge until the corpuscular mass appears translucent: the lengths of the columns of corpuscles and plasma are then measured off by laying the tubes on a scale, and the mean of the two readings taken, the corpuscular volume being expressed as a percentage of that of the whole blood. This method eliminates the error due to taper of the capillaries; the following duplicate percentage figures show how great that error might otherwise be.

	First pair			Second pair		
Blood of	A	B	Mean	A	B	Mean
Anæmic rabbit	20	16·9	18·5	19·3	17·6	18·5
Dog	38·8	36·6	37·7	41·3	33·6	37·5

I. The regulation of the reaction of plasma.

The inorganic constituents of plasma. We owe to a large number of investigators, but especially in recent times to L. J. Henderson(18), Hasselbalch(16), Cullen(5), Michaelis(21), and Bayliss(2), the opinion that sodium bicarbonate is, if not the only one, at least the chief buffer of the blood plasma. The conclusions from my own experiments, while not at variance with this view, have nevertheless convinced me that there are well marked differences between plasma and bicarbonate solutions of equivalent bicarbonate content as regards their reaction at

a given pressure of carbon dioxide; differences, in fact, as great as *p*.H 0·3 (cp. p. 170). The principal difference between a plasma and a bicarbonate solution of the same alkali reserve is that due to the sodium chloride contained in the former; the effect of an addition of sodium chloride on the R-CO_2 curve of a bicarbonate solution is illustrated by Fig. 1. As shown by Milroy(24), the solution of bicarbonate to which chloride has been added is at each pressure of carbon dioxide more acid

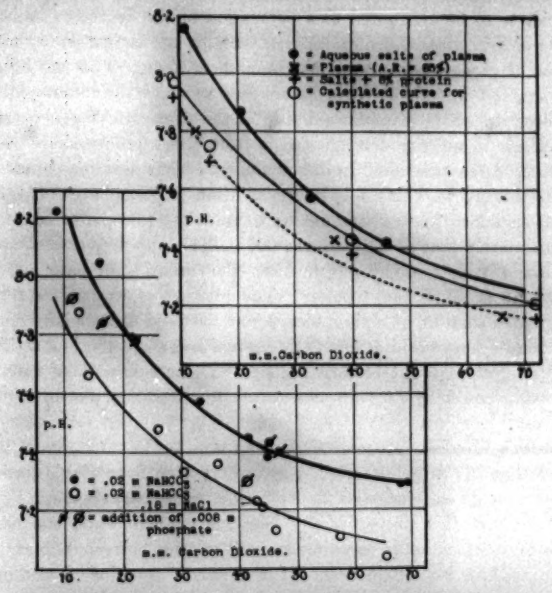

● = Aqueous salts of plasma
⊗ = Plasma (A.R.P. 60%)
+ = Salts + 6% protein
○ = Calculated curve for synthetic plasma
p.H.
m.m.Carbon Dioxide.
● = .02 m NaHCO3
○ = .02 m NaHCO3 .16 m NaCl
Ø = addition of .008 m phosphate
p.H.
m.m. Carbon Dioxide.

Fig. 1. Lower figure. Fig. 2. Upper figure. For description see text.

than that without such addition. As Milroy used the hydrogen electrode it is certain that this difference is not due to some error in the colorimetric method, occasioned by the salt added. The phosphates of plasma, which are the only other salts of note, are, according to the analyses of Abderhalden(1), only present to the extent of about ·007–·008 p.c. (say ·008 molar). The quantity determined by Cushny(6) in the colloid-free filtrate of serum was of the same order.

In Fig. 1 the upper curve is that for ·02 molar (·168 p.c.) sodium bicarbonate in water (actual alkali reserve = 43·5 p.c.), and the lower one that for the same solution containing in addition ·18 molar (1·06 p.c.) sodium chloride: the solution is therefore somewhat weaker in bicarbonate, and considerably stronger in sodium chloride than is the plasma of ordinary blood (which usually contains about ·6 p.c. of NaCl and ·2–·25 p.c. of $NaHCO_3$), and shows the effect of the chloride in a correspondingly exaggerated form. In all probability the effect of the sodium chloride is to be attributed to a depression of the degree of ionisation of the bicarbonate. Calculation from such curves indicates(10) that the degree of dissociation of the bicarbonate is about ·9 in the aqueous solution, and only about ·57 in the solution to which the sodium chloride has been added. Potassium chloride in the same molar concentration has effects in no way distinguishable from those produced by sodium chloride. A few points on the curves indicate determinations made with these solutions to which a balanced solution of phosphates of *p*.H 7·5 had been added in such amount as to raise the phosphate content to approximately that of the plasma (·008 molar); but they do not appear to show any noteworthy effect, when the quantity of base added as phosphate is taken into account. The relation of the points for the phosphated solution of sodium bicarbonate to those of the plain aqueous bicarbonate (more acid at low CO_2 tensions and more alkaline at higher ones) suggests a slight buffering effect, such as was demonstrated for CO_2-fixing power by Straub and Meier(31) by adding phosphoric acid to serum.

Plasma proteins. Moore, McQueen and Webster(25) have maintained that the proteins function as buffers even over viable limits of carbon dioxide concentration and reaction, while Bayliss(2) has given evidence that this property only becomes evident when the carbon dioxide concentration is very low, and the solution correspondingly very alkaline. The isoelectric point of the serum proteins according to Michaelis(21) is in the neighbourhood of *p*.H 5, and this, together with Hardy's demonstration(15) that in serum globulin the acid functions are more pronounced than the basic ones, would lead one to expect that, at the ordinary reactions of blood plasma, the serum proteins might function as acids, and compete with carbon dioxide for possession of the base. In order to ascertain the influence of the proteins on the reaction of plasma, some plasma was separated from horse blood at 19° C. and 66 mm. carbon dioxide. This plasma had the following composition:

Alkali reserve 85 p.c. = ·038 m.
Chloride as NaCl ·595 p.c. = ·102 m. } Na as chloride and bicarbonate = ·14 m.
Protein = 8·08 p.c. (1·273 p.c. nitrogen).
Total solids 9·0 p.c. Sulphated ash = 1·09 p.c., *i.e.* base as Na = ·154 m.

It is perhaps worthy of note that the total base calculated as Na is ·014 m. greater than that present as chloride and bicarbonate: if we allow for the presence of one-twentieth of the base being present as potassium, this would reduce the discrepancy to about ·009 m., an amount which is practically identical with that present as phosphate according to **Abderhalden**. There seems, therefore, to be no margin left for combination with proteins at 40 mm. carbon dioxide, the tension at which the alkali reserve was determined.

The R-CO_2 curve of this plasma was plotted at 19°, and the portion of the curve between 10 and 70 mm. CO_2 is shown by the dotted line of Fig. 2 (the whole curve is not drawn). Artificial mixtures were now made to imitate this plasma, and to begin with the inorganic salts, in aqueous solution, were taken (phosphate being omitted). This solution had the composition $NaHCO_3$ ·038 m.; NaCl, ·1016 m.; KCl, ·0004 m. (the identical effect of KCl and NaCl had not at that time been established). The R-CO_2 curve of this solution is shown by the upper line of Fig. 2. The curve runs practically parallel with that of the plasma, but definitely higher. A plasma and a bicarbonate solution, of identical bicarbonate content, therefore have different reactions at the same carbon dioxide pressures, though, as judged by the slope of the curves, both are uniformly buffered. Presumably the difference is due to the content of protein. Now, in 50 c.c. of the saline solution were dissolved 1 gram of pure serum albumin and 3 grams of pure serum globulin. These proteins, prepared by Dr H. W. Dudley, were of a high degree of purity and had been subjected to prolonged dialysis. The R-CO_2 curve of this "synthetic" plasma was also plotted at the same temperature, and some of the points are indicated by the vertical crosses of Fig. 2. At first sight the agreement with the curve for the original natural plasma appears to be satisfactory enough: the curve falls distinctly below that of the aqueous solution of salts. But when the alkali reserve of this synthetic plasma was determined it was found to be only 70 instead of 85 p.c. From the results of experiments on other plasmas, which will be detailed later, it was possible to allow for this difference of alkali reserve, and when this is done, the curve for the synthetic plasma, had its alkali reserve been 85 p.c., would now fall on the points shown by the middle curve cf Fig. 2. This curve is still below that for the aqueous salts, but well above that for the original plasma: the effect of the dried proteins employed nevertheless seems to differ from that of the natural plasma proteins.

The fact that the alkali reserve of a bicarbonate solution is lowered

by the addition to it of protein has also been noted by Hasselbalch(16), and I therefore repeated the experiment with sodium bicarbonate. The protein was dissolved in and made up to a given volume with the bicarbonate solution.

Exp. 2.	$NaHCO_3$ p.c.	Serum globulin p.c.	Alkali reserve	p.H at 42 mm. Hg. CO_2
	·336	0·0	88·0	7·67
	·336	8·0	81·5	7·52

A somewhat similar effect has been observed by Bayliss (*l.c.*), and attributed by him to the volume taken up by the dissolved protein. The only satisfactory way of making up such a solution would appear to be to dissolve the solids together and make up to a known volume. This was done in Exp. 3.

Exp. 3. Sodium chloride and sodium bicarbonate were dissolved in water and made up to a known volume: the solution contained approximately ·04 m. $NaHCO_3$ and ·14 m. NaCl. A similar solution was made up with addition of 8 p.c. of pure serum globulin before making up with water to the known volume. In the course of solution of the protein in the bicarbonate solution, small bubbles of gas were slowly evolved, but the amount of gas (presumably CO_2) so generated was very small. A third solution of NaCl and $NaHCO_3$ was made up to have the same alkali reserve as was found by experiment in the second solution. The reactions of these solutions at 42 mm. CO_2 were determined, and that of a plain aqueous bicarbonate of the same alkali reserve as the protein-containing solution (ascertained from Fig. 5) is included in the table.

NaCl molar	$NaHCO_3$ molar	Protein p.c.	Actual alkali reserve p.c.	p.H at 42 mm.
·14	·04	—	90	7·47
·14	·04	8	83	7·33
·14	·037	—	83	7·37
0·0	·037	—	83	7·68

It appears from these results that the protein had expelled a small amount of carbon dioxide from the bicarbonate, and that the reduction of the alkali reserve was due to this. When bicarbonate chloride solutions with and without protein and of the same alkali reserves are compared, the difference in reaction is much smaller (·04), and probably within the limits of experimental error. The difference between bicarbonate solution and plasma then is almost entirely due to the presence in the latter of chlorides, and the proteins show no buffering properties. This view is further supported by Cushny's finding that the difference in inorganic salt content between plasma and its colloid-free filtrate is almost or quite negligible; also by Cullen's experiments(5) on the electrometric titration of plasma.

Hasselbalch and Warburg(17) have shown that with various other ampholytes such as glycine, histidine or hæmoglobin, the carbon dioxide fixing power is at p.H above the isoelectric point, less, and below it

greater, than that of a bicarbonate solution in which they are dissolved. The isoelectric *p*.H point of serum proteins is certainly below that for any reasonably high CO_2 concentration. If the plasma proteins do seize on any small quantity of base, they evidently hold it so firmly that the only effect of their presence is very slightly to reduce the effective concentration of bicarbonate in the solution: another explanation of the difference between the curve for the plasma and that for its salts would be that bicarbonate is adsorbed by the protein, but can be expelled again by strong acids, as in the determination of the alkali reserve.

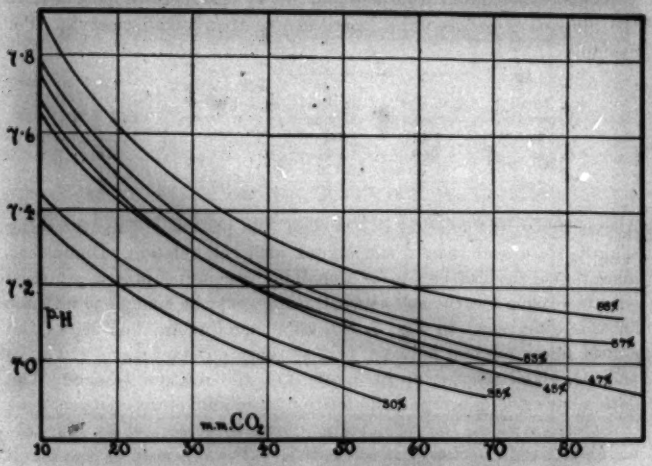


Fig. 3. R-CO_2 curves at 20° for plasmas from different sources; the alkali reserves are shown by the numbers on the curves.

Whatever the explanation may be, one thing seems certain, that the buffering power of a plasma is, for practical purposes as claimed by Bayliss, not greater than that of a corresponding plain bicarbonate solution, and appears to be distinctly less.

The reaction curves of plasma from different sources. The R-CO_2 curves for seven different plasmas of alkali reserves ranging from 30 to 85 p.c. are given in Fig. 3. The determinations were made at 18–20° C. The most striking feature of these curves is that they are all roughly parallel one with another. Two of the curves are of plasmas from the same horse blood, and three of them from the same rabbit blood, the specimens being

drawn off at different carbon dioxide tensions. Now Hasselbalch and Warburg (*l.c.*) have shown that the CO_2 content-tension curves of sera of different bicarbonate content also run parallel with one another, and it therefore seems reasonable to expect that there would be a direct relation between the alkali reserve of a plasma and its H-ion concentration at any given pressure of carbon dioxide. Figs. 4 and 5 show that this is approximately true. The curves in Fig. 4 are calculated from those of Fig. 3, and express the results in increments of 10 p.c. in the alkali reserve, from 30 to 80 p.c. The curves are, as would be anticipated, separated from one another by distances proportional to the differences between the logarithms of their alkali reserves. For example

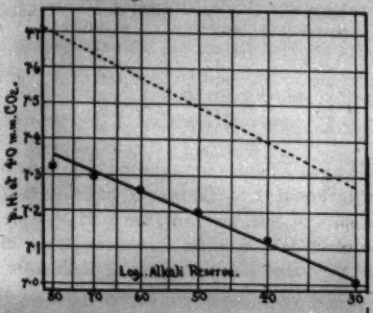
TABLE I.

Alkali reserve	Log. alkali reserve	p.H at 40 mm.	Diff. between log. alkali reserve	Diff. p.H
30	1·48	7·0	—	—
40	1·60	7·12	·12	·12
60	1·78	7·26	·18	·14
84·5	1·93	7·33	·15	·07

Above 60 p.c. the relation is less close, the plasma apparently being slightly more acid than would accord with this relation. Diminished ionisation of the bicarbonate (or a small buffer action of plasma proteins) with increasing bicarbonate concentration would be enough to account for this difference. In Fig. 5, the lower, continuous, line shows the almost linear relation between p.H at constant CO_2 tension (40 mm.) and log. alkali reserve: up to 70 mm. CO_2 the relation is good. The dotted line represents the similar relation for sodium bicarbonate solution in water, drawn on the assumption (shown to be correct by Hasselbalch *l.c.*) that, as for plasma, the c.H varies inversely as the alkali reserve. It is seen to run almost parallel with the plasma curve, but is, at 40–50 mm., about p.H 0·3 higher. We may conclude from these experiments that the reaction of a plasma is determined by its bicarbonate-CO_2 ratio as found by Hasselbalch, but it is important to observe that there is such a wide discrepancy between the actual reactions of plasmas and bicarbonate solutions of the same alkali reserves, as cannot possibly be due to an ordinary experimental error (Fig. 5). The differences (due to chlorides and proteins) are however constant, not only, as already shown, for different pressures of carbon dioxide, but also for different alkali reserves. Since the reaction of bicarbonate-holding fluids, as determined by the hydrogen electrode, is too acid by about p.H 0·2, and since other salts alter the reaction of a bicarbonate

solution, the method introduced by Hasselbalch (*l.c.*), of calculating the $p.H$ of bicarbonate solutions from the results of determinations of the carbon dioxide and bicarbonate ratio, cannot be applied directly to plasma or blood. But the method could be applied quite easily if the

Fig. 5.



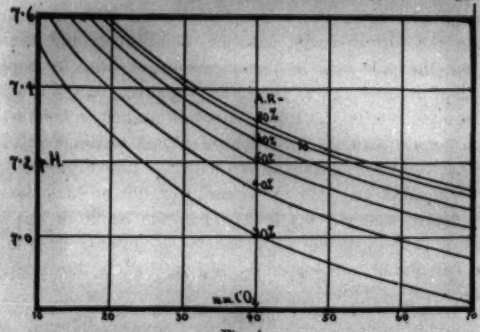


Fig. 4.

Fig. 4 (lower). Constructed from the data of Fig. 3. Curves for plasmas ascending in alkali reserve by 10 p.c.

Fig. 5 (upper). Linear relation between log. alkali reserve and $p.H$ at 40 mm. CO_2 for plasma (continuous line) and for aqueous bicarbonate solution (dotted line).

values for the constant $p.K_1$ in the formula $p.H = p.K_1 + \log \frac{[NaHCO_3]}{[CO_2]}$ were calculated under the conditions which obtain in plasma, and as the curve of Fig. 5 supplies the necessary data, I have calculated some values for $p.K_1$. The results are given in Table II to three significant

figures, the values for 38° being calculated by the addition of ·11, in accordance with observations of Hasselbalch (*l.c.*) on the effect of rise of temperature on the reaction of bicarbonate solutions.

TABLE II.

Alkali reserve	Values of $p.K_1$ for plasma at 20°	at 38°	Hasselbalch's value for $p.K_1$ at 38°
30	6·20	6·31	6·41
40	6·17	6·28	6·39
50	6·16	6·27	6·37
60	6·14	6·25	6·36
70	6·13	6·24	6·355
80	6·12	6·23	6·35

Hasselbalch's figures for aqueous bicarbonate solutions are *higher* than mine by about ·1 to ·12, instead of being ·2 lower as I had anticipated. In spite of this, however, the $p.H$ of a plasma calculated with Hasselbalch's data is, like that determined electrometrically, 0·2 *lower* than the value determined with indicators. The divergence is explained in the following way. In consequence of the differences between plasma and bicarbonate (see Fig. 5), the latter has a $p.H$ 0·3 higher than the former under identical conditions; and therefore to deduce, from the $p.H$ of a bicarbonate solution, that for a plasma of the same alkali reserve and at the same CO_2 pressure, 0·3 must be subtracted. In other words, the total correction needed to convert electrometric bicarbonate readings to indicator plasma readings will be about (+ ·2—·3) *i.e.* minus 0·1, which agrees with the above values for $p.K_1$. To put it in another way, the electrometric result on bicarbonate solution is 0·1 *higher*, and not 0·2 lower, than the result of the indicator method for a plasma of the same $NaHCO_3$ content. In the original method of calculation advocated by Hasselbalch, however, he uses the formula

$$p.H = p.K_1 + \log \frac{(\text{alkali reserve})}{2 \times \frac{1}{1.1} p.a} = p.K_1 + \log \frac{3{\cdot}8 \text{ A.R.}}{p.a}$$

as a result of which the carbon dioxide concentration appears twice as great as is actually the case, and therefore in his formula the value of the last term $\log \frac{(NaHCO_3)}{(CO_2)}$ is too low by $\log 2$, *i.e.* by 0·3. This anomaly has already been pointed out by Parsons(27), who suggests that the values of $p.K_1$ should be calculated in terms of molecular quantities, and the correct expression for the bicarbonate-CO_2 ratio used. Parsons' suggested modification would correct the second term but would balance the correction by making $p.K_1$ 0·3 too low. In either case the result would happen to correspond with the determination made on plasma

by the hydrogen electrode method, being 0·2 below the (presumably correct) value as determined by the use of indicators.

Now, if we take, instead of Hasselbalch's values for $p.K_1$, or those proposed by Parsons, the values given in the preceding table, and instead of his formula the more probable one

$$p.H = p.K_1 + \log \frac{7{\cdot}6 \text{ A.R.}}{p.a}$$

we find that there is reasonably good agreement between the results of the modified calculation and those of the indicator determination of hydrogen ion concentration (this applies only to plasma; blood will be considered later). The following table gives the results of such calculations, from two of the plasmas chosen at random.

TABLE III.

Plasma of rabbit. Alkali reserve = 45 p.c. $p.K_1 = 6{\cdot}17$ at 20°.

CO_2 pressure mm.	$p.H$ calculated	$p.H$ found	Difference
10 (interpolated)	7·75	7·72	−0·03
18·5	7·48	7·48	±0·00
33·5	7·24	7·25	+0·01
50 (interpolated)	7·05	7·10	+0·05
76·5	6·87	6·95	+0·08

Plasma of rabbit A.R. = 57 p.c. $p.K_1 = 6{\cdot}15$.

21	7·51	7·47	−0·04
38·5	7·24	7·23	−0·01
56·5	7·08	7·11	+0·03
74·0	6·95	7·02	+0·05

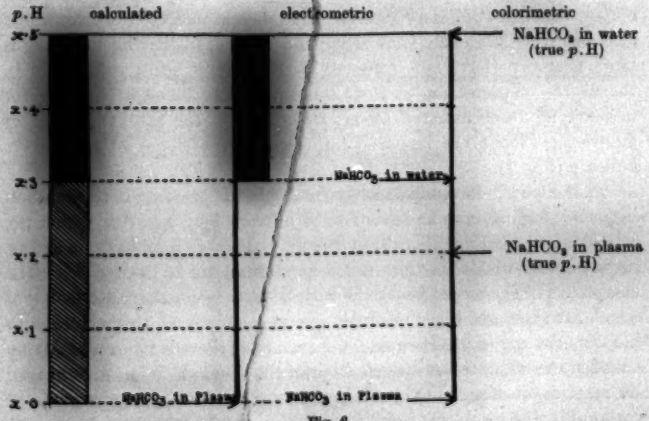


Fig. 6.

These relations are given in the form of a diagram in Fig. 6. This shows the relation between the p.H of plasmas and bicarbonate solutions of the same alkali reserve, as determined by calculation with Hasselbalch's original data and formula, by the hydrogen electrode, and by means of the indicator method. The area shown in black in the figure represents the electrode error: that which is shaded represents the discrepancy introduced in the last term of the Hasselbalch formula. Calculation with the new values for $p.K_1$ here given and with the simpler formula would, of course, give the same results as the indicator method.

II. The regulation of the reaction of blood.

The carbon dioxide reaction curve of blood. When we turn to the consideration of the reaction curves for whole blood, it is at once apparent that we have to deal with a much more complex state of affairs than obtains for separated plasma; for, owing to the presence of the corpuscles, the bicarbonate concentration of the plasma, on which, as we have seen, its reaction at a given carbon dioxide concentration depends, is itself variable with the concentration of carbon dioxide in the blood. If the alteration in the bicarbonate concentration of the plasma were exactly proportional to the change in its carbon dioxide concentration, then the reaction of blood would be the same at all carbon dioxide pressures—it would be perfectly buffered. This is not the case, though the bicarbonate concentration does increase with increasing CO_2 pressure: blood thus behaves differently from separated plasma, in that its buffering is more nearly perfect, but different bloods exhibit great differences in the degree of perfection of their buffering. The blood of the dog for instance is a well-buffered blood: so is that of the rabbit: human or guinea-pig bloods are less well buffered, as the slopes of the curves indicate (Fig. 7).

That blood behaves differently from plasma as regards the relation between alkali reserve and reaction, is indicated by Fig. 8, in which the relations between log. alkali reserve and p.H at 40 mm. CO_2 are shown for a number of bloods from different sources, at 20° and 38° C. The complete R-CO_2 curves from which these data were obtained (some of which are shown in Fig. 7) differed enormously among themselves both in steepness and in position, and it is seen that there is no exact relation between the alkali reserve (fixed CO_2) of the whole blood at 42 mm. and its reaction at 40 mm. CO_2, while the reactions at lower pressures of carbon dioxide would bear still less relation to the CO_2 fixed at 42 mm.

If it were possible to draw curves at all, they might have some such form as those indicated by the dotted lines of Fig. 8 though not much stress can be laid on this. It is sufficient to say that any relation shown is of the roughest, and that it is not linear. It might be objected, and with justice, that in determining the "alkali reserve" of the blood (which was done at 42 mm.), we have no measure of the total reserve of base held by the corpuscles; but since the *p*.H chosen is that at 40 mm., this probably matters very little for our present purpose: the curves, if they mean anything at all, show that as the alkali reserve of

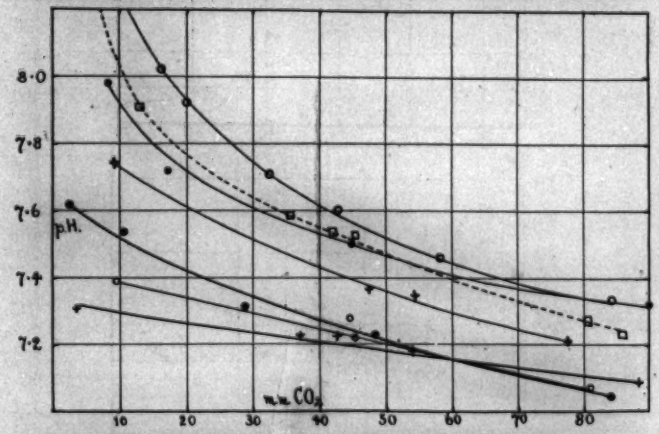


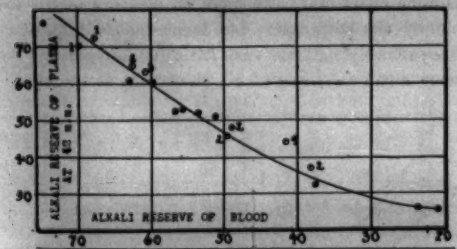
Fig. 7. R-CO_2 curves of bloods at 38° C. In order from above downwards (left-hand ends) the curves are for bloods of 67, 60, 65, 58, 38, 43 and 24 p.c. alkali reserve, from goat, man, guinea-pig, horse, rabbit, dog and rabbit respectively.

the blood is lowered, the fraction of the carbon dioxide fixed by the plasma is increased, or, what amounts to the same thing, the fraction of the fixed carbon dioxide which is carried by the corpuscles diminishes with diminution of the total fixed carbon dioxide, a fact which has also been demonstrated by Joffe and Poulton[19] and Haggard and Y. Henderson[13] in other ways, and to which reference will be made again later. Similar conclusions are to be drawn from another type of experiment (Fig. 9) in which the alkali reserves of different plasmas and the corresponding bloods, separated at 42 mm., are compared. The graph shows similar relations between the alkali reserves of plasma and

whole blood, to those shown in Fig. 8, though the curvature is less marked in the present instance.

Another possible explanation of the irregular relation between the reaction and carbon dioxide content of different bloods is that, in many

Fig. 9.



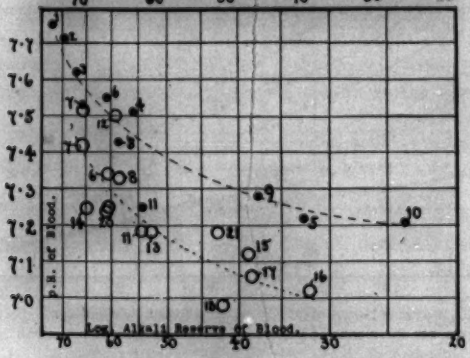


Fig. 8.

Fig. 8 (lower). Irregular relation between blood CO_2 content and reaction at 40 mm. for comparison with Fig. 5. Black points are determinations at 38° and circles at 20° C.

Fig. 9 (upper). Relation between "alkali reserve" (CO_2 content) of whole oxalated blood at 42 mm. and alkali reserve of plasma drawn from it. Nos. 1–4 for blood of goat, cat, man and dog respectively. The remainder for the rabbit.

instances, the bloods have very different hæmoglobin contents, or even (what is quite probable) that the hæmoglobins of different animals have different buffering powers when presented with identical CO_2 concentrations. Suppose now that bloods from the same individual, of the same corpuscle content, but of different alkali reserves are compared,

would these show a linear relation between p.H and log. alkali reserve, or would the graph have a similar curvature to that shown in Fig. 8? Fig. 10 shows that the latter is the case. The experiment was carried out with the same goat's blood. Slight variations in alkali reserve, such as are common in all herbivora, appeared in the blood collected at different times, but greater changes in the available base were induced by addition of HCl or $NaHCO_3$ to the blood. In this way the alkali reserve was altered from 56 p.c. to 33 p.c. and to 72 p.c. The explanation is as before, that when the total fixed carbonic acid is low a slightly

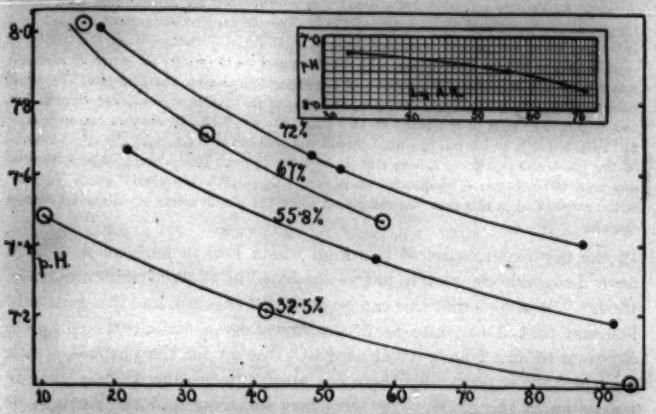


Fig. 10. Effect of alteration of alkali reserve of the blood of the same goat, 38° C. The figures above each curve show the alkali reserve of the blood. Small figure inset: relation between log. alkali reserve and p.H at 40 mm. (p.H ordinates number from above downwards.)

greater fraction of it is carried in the plasma, while when it is high, a somewhat greater proportion is carried by the corpuscles.

The rôle of the corpuscles. It is clear that, if we consider the buffering of drawn plasma as simple, or direct, we must regard that of the plasma of whole blood as indirect or secondary. This of course is a distinction which is made only for the sake of convenience, and refers to the plasma of blood exposed to appreciable carbon dioxide tensions. Clearly, when the pressure of carbon dioxide is reduced to zero, the potential buffering of the blood is almost entirely secondary. Indeed, if there were absolutely no union between the plasma proteins and the residual base of

the plasma to form hydrolysable salts, the plasma should be neutral in reaction, as soon as it was free from carbonate, the available base being all taken up by HCl liberated from the corpuscles. The fact that the plasma under these circumstances is always strongly alkaline may be taken as an indication of the presence of plasma-base salts, under conditions such as were indicated by Bayliss(2). The plasma, which is drawn from blood when it is eventually freed or nearly freed from carbonate, still holds plenty of available base in this form, as the following experiment shows:

Exp. 4. Freshly oxalated cat's blood was equilibrated for 90 minutes with atmospheric air, renewed each 20 minutes, at 17° C. The carbon dioxide content of the blood, and also that of the plasma separated from it, was 7·4 p.c. Another sample of the blood equilibrated at 38° for three hours, with similar renewals of (moist) air every 20 minutes, had a p. H of 8·64. The alkali reserve of the separated plasma was 27·5 p.c. The alkali reserve of the whole blood was 49·7 p.c., and that of its plasma separated at 42 mm. CO_2 was 46 p.c. The p. H of the blood at 44·5 mm. CO_2 was 7·13. In another instance after 2½ hours, the CO_2 content of the whole blood was reduced to 5 p.c., and the alkali reserve of the plasma drawn from it was still as high as 19 p.c. The potential alkali-forming power of 100 volumes of corpuscles at 42 mm. CO_2 (*i.e.* the so-called "alkali reserve" of the corpuscles) in this case was calculated to be 111 p.c. in terms of volume of carbon dioxide.

In the few experiments of this kind which I have performed, I have never been able completely to free the blood of all fixed carbon dioxide, though it is known that this can be done in a vacuum, and it is generally believed that it can also be accomplished by a sufficiently complete exposure to air. I have never used CO_2-free air for the purpose, which may perhaps explain why there has always been some carbon dioxide remaining in the blood. The secondary buffering and the carriage of carbon dioxide by the red corpuscles probably supply an explanation for the fact that the alkali reserve (and p. H) of the plasma drawn from a blood varies so much in different instances, and bears no simple relation to the total CO_2-fixing power of the whole blood. The great alkali-producing power of the corpuscles has been known for a very long time, but even at the present time is scarcely recognised as one of the most important of the properties of the blood, which it undoubtedly is (Zuntz(34), Hamburger(14), Van Slyke and Cullen(33), Fridericia(11), L. J. Henderson(18), Haggard and Yandell Henderson(13)).

Another property of blood to which reference is often made in this connection (Parsons(26)) is the fact, also suspected by Zuntz (*l.c.* p. 81), but first clearly shown by Christiansen, Douglas and Haldane(4), that the CO_2-fixing power is less in oxygenated than in reduced blood. This is generally accepted as an indication that oxy-

hæmoglobin is a stronger acid than hæmoglobin. This difference between oxygenated and reduced blood is associated with a lower H-ion concentration at a given CO_2 pressure and with greater differences in the magnitude of the chloride and bicarbonate shifts produced by a given increase of carbon dioxide pressure in the reduced than in the oxygenated blood, as the following example illustrates.

Exp. 5. Horse blood. Corpuscular volume = 28·9 p.c. Oxygen capacity = 11·5 p.c.

(*a*) *Points for* R-CO_2 *curve.*

	mm. CO_2 at 20° C.									
	10	20	30	40	50	60	70	80	90	100
p.H reduced blood	7·76	7·58	7·47	7·40	7·32	7·25	7·19	7·13	7·08	7·04
p.H oxygenated blood	7·76	7·52	7·41	7·33	7·26	7·20	7·14	7·10	7·05	7·01
Difference...	0·00	0·06	0·06	0·07	0·06	0·05	0·05	0·03	0·03	0·03

(*b*) *Change in composition of plasma.*

Partial pressure of gases to which blood was exposed. mm. Hg.			Composition of plasma drawn at gas pressures given in columns 1 and 2	
	CO_2	O_2	Chloride, as NaCl p.c.	Alkali reserve
(Reduced)	1·0	5·5	·627	40·3
,,	95·0	4·8	·585	84·4
(Oxygenated)	6·0	736	·615	44·3
,,	91·0	651	·585	78·6

(*c*) *Shift of* Cl *and* CO_2 *for equal change of* CO_2 *pressure (calculated from* (*b*)).

	Change in p.c.		Change in molarity		
	Oxygenated	Reduced	Oxygenated	Reduced	Difference
Cl, for 85 mm. CO_2	−·030	−·038	−·005	−·0068	−·0018
A.B., ,, ,,	+34·3	+39·9	+·0156	+·0178	−·0022

Inspection of these figures shows (1) that oxygenated blood is more acid than reduced blood by an average of *p*.H ·045: this figure does not differ greatly from that found by Parsons (26) by an indirect method, for human blood at 38°, viz. ·038; (2) the shifts of Cl and HCO_3 are not equi-molar, a fact already noted by Van Slyke and Cullen (*l.c.*); (3) the shift, both of Cl and of CO_2, is greater for reduced than for oxygenated blood by about ·002 molar.

Since, in the above example, 28·9 volumes of corpuscles yield, to 71·1 vols. of plasma, 34·3 and 39·9 vols. respectively of carbon dioxide, it can be calculated that the base-liberating power (or HCl-fixing power) of 100 vols. of corpuscles, is, for the same change of 85 mm. CO_2, equivalent to 84 vols. of CO_2 for oxygenated blood, and 98 vols. for reduced blood. The actual amount of base, as Na, set free by this 100 vols., would be about 86 and 100 mg. respectively. Now, from the oxygen capacity of the corpuscles, the amount of hæmoglobin in them is about

29·8 p.c.: if we take the molecular weight of hæmoglobin as 17,000, and regard it as a monobasic acid, the amount of base fixed by 100 vols. of corpuscles containing 29·8 grams of hæmoglobin would be only about 40 mg. That is to say, the corpuscles are about ·018 molar in hæmoglobin, but have effected an alkali generation equal to about ·04 molar under the present conditions: if the plasma had been drawn off at zero pressure of CO_2, at which we may suppose it to have as in Exp. 4 an alkali reserve of 20 p.c., the base yielding power of the corpuscles would have been at least half as great again for a change to 95 mm. CO_2, *i.e.* it would have been about ·06 m. Campbell and Poulton(3) give the maximal available base of the whole blood in man as ·03 normal, and

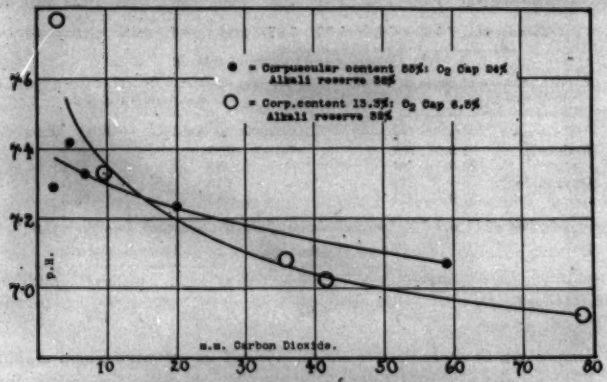


Fig. 11. Alteration of corpuscular content.

as the blood they used had a corpuscular volume of about 50 p.c., this also would represent about ·06 m. as the alkali generating power of human corpuscles. There are three ways in which the disparity between hæmoglobin content and buffering power of the corpuscles may be interpreted, viz. (1) that the molecular weight of hæmoglobin is much less than 17,000, and nearer to 5000, which seems impossible; (2) that hæmoglobin is polybasic (or polyacid) of valency 3; or (3) that there is some other mechanism determining the secondary buffering, in addition to the hæmoglobin. It would be unprofitable to discuss these alternatives at present, though the second would seem to be the most likely.

The influence of corpuscular volume. The total buffering effect of the corpuscles would be expected to be greater when their volume was

greater, and experiment shows that this is the case: when the corpuscular content is increased the curve is a flatter one. This is indicated to some extent by the curves for different animals given in Fig. 6, but the issue is complicated by the difference in species. In one experiment I altered the corpuscular content of cat's blood by centrifuging off some plasma from one portion and adding it to the other portion (Fig. 11). Unfortunately the alkali reserves of the two specimens which were thus obtained were slightly different, but the alteration in the form of the reaction curve is very plain.

The alkali reserve of the corpuscles. If we determine the fixed CO_2 content of whole blood and of its plasma at a definite pressure of CO_2, say 42 mm., and also know the volume of the corpuscles present, we can determine how much of the fixed CO_2 of the blood is carried by the corpuscles, at that pressure of CO_2. I have often, for convenience, designated this the alkali reserve of the corpuscles: it can be expressed in terms of the carbon dioxide carried, either by 100 vols. of corpuscles, or by the corpuscles of 100 vols. of blood. It does not mean the total available base of the corpuscles, but the amount of carbon dioxide carried by and in the corpuscles at 42 mm. CO_2 pressure.

Exp. 6. Cat's oxalated blood. Corpuscular vol. = 27·6 p.c.

Plasma separated at. mm. CO_2	Distribution of CO_2 in 100 vols. blood		CO_2-fixing powers of plasma and corpuscles of 100 vols. blood at 42 mm.		
	Corpuscles	Plasma	Corpuscles	Plasma	Total
0·5	2	5	28	17	45
44·5	16	33	16	33	49
Exp. 7. Horse blood. Corpuscular vol. = 39·8 p.c.					
3·8	—	—	33	32	65
66·0	—	—	14	51	65

These results show that as with increasing CO_2-pressures the amount of carbon dioxide carried separately by the corpuscles and by the plasma increases, the CO_2-fixing reserve of the corpuscles is reduced (*i.e.* their acid load, Haggard and Henderson(13), is increased) while that of the plasma is increased. The higher content of carbon dioxide in the corpuscles at higher pressures is only explicable on the hypothesis that the cells are penetrable, not only by HCl, but also by carbonic acid, and that their contents can fix this substance either as bicarbonate or in union with the hæmoglobin. The following experiment shows that at the same CO_2-pressures, the CO_2-fixing power of corpuscles and plasma are mutually interdependent.

Exp. 8. To portions of centrifuged oxalated cat's corpuscles suspended in their own volume of saline solutions of equivalent normality HCl or $NaHCO_3$ was added as in col. 1 of the table. These were equilibrated with alveolar air (42 mm. CO_2) and the total fixed CO_2 of suspension, corpuscles and fluid determined.

Solution added in equal volume to corpuscles	Fixed CO_2 of 100 vols. suspension		Fixed CO_2 of 100 vols. corpuscles		Fixed CO_2 of 100 vols. saline	
1 = ·124 m. NaCl	—		—		—	
·02 m. HCl	22	15	21	14	22	18
2 = ·144 m. NaCl	37		35		40	
3 = ·124 m. NaCl	—	13	—	3	—	18
·02 m. $NaHCO_3$	50		38		58	
4 = ·104 m. NaCl	—	14	—	10	—	18
·04 m. $NaHCO_3$	64		48		76	

When, by addition of acid or bicarbonate to the suspension, the acid load of the corpuscles is increased or diminished, the alkali reserve of the mixture is also lowered or raised, but not, as might have been expected, by 22 vols. p.c. (·01 m.), but only by 15, 13 and 14 vols. p.c. respectively. Of the alterations effected by change in the composition of the suspending solution, the most regular are those of the supernatant fluid (18 vols. in each case). The corpuscles were most affected by the addition of acid: alkali had less effect, perhaps because it did not penetrate the cells. When the alkali reserves of the suspension are plotted against those of the supernatant salines, the graph shows a similar curvature to those reproduced in Figs. 8 and 9. A simple way of regarding these results is to consider that the corpuscles take up acid from or yield acid to the saline fluid according to the requirements, the net result being that the effect of addition of either acid or alkali in known amount is less than would be calculated if no secondary buffering took place.

The mutual relations between corpuscles and plasma also emerge, though rather less distinctly, when one tabulates the fixed CO_2 of plasma and corpuscles at 42 mm. for different bloods. The corpuscular volume, which also influences the result, is added in Table IV.

From this table, the following facts appear more or less clearly:

(1) The proportion of carbon dioxide fixed by unit volume of plasma and corpuscles increase or decrease together, but not in strict proportion.

(2) For the same species, the CO_2 fixing power of the corpuscles present in 100 vols. of blood tends to vary from a certain number (24 for the goat, 14 for the rabbit, 18 for man).

(3) The greater the corpuscular volume, the smaller is the volume of CO_2 fixed by unit volume of corpuscles, but the greater the total amount which the corpuscles carry.

(4) In consequence of this, the higher the corpuscular content, the greater the alkali reserve of the plasma compared with that of the corpuscles. That is to say, a given volume of corpuscles has, other things being equal, a greater alkali generating power when suspended in a large than when suspended in a small volume of fluid: this has also been demonstrated by Haggard and Henderson(13), who suggest as the explanation that the intensity of the alkali production when corpuscles

TABLE IV.

(*a*) *Data arranged in order of descending alkali reserves of plasma.*

Animal	Corpuscular volume	CO_2 fixed per 100 vols. of blood	CO_2 fixed per 100 vols. of plasma	CO_2 fixed per 100 vols. of corpuscles	CO_2 fixed by corpuscles of 100 vols. blood
Rabbit	19·6	75	77	66	13
Goat	39·8	68	73	59	24
Goat	35·1	70	70	70	24
Human	33·1	60	63	54	18
Rabbit	26·0	60	61	57	15
Rabbit	20·8	57	53	71	15
Rabbit	22·0	56	53	66	15
Rabbit	22·5	53	52	50	13
Cat	24·0	49	48	50	12
Dog	37·5	41	44	37	14
Cat	31·0	38	37	42	13

(*b*) *Data in order of ascending corpuscular volumes.*

Animal	Corpuscular volume	Alkali reserve of plasma	Alkali reserve of corpuscles	Greater A.R. is in
Rabbit	17·9	60	74	corpuscles (14)
Rabbit	20·8	53	71	corpuscles (18)
Rabbit	22·0	53	66	corpuscles (13)
Rabbit	22·5	52	59	corpuscles (7)
Cat	24·0	48	50	corpuscles (2)
Rabbit	26·0	61	57	plasma (4)
Cat	31·0	37	42	corpuscles (5)
Human	33·1	61	57	plasma (4)
Rabbit	33·7	51	51	equal (0)
Goat	35·1	70	70	equal (0)
Dog	37·5	44	37	plasma (7)
Goat	39·8	73	59	plasma (14)

The figures in brackets in the last column indicate the difference between corpuscles and plasma, in p.c. CO_2 fixing power.

are exposed to CO_2 is determined by the rise of the *c*.H of the plasma, and will obviously be proportionally more per unit volume of corpuscles when that unit volume is suspended in a large volume of saline, because there is a smaller mass of corpuscles to produce alkali and so to prevent the H-ion concentration from rising in the plasma.

Alteration in the carbon dioxide-fixing power of the corpuscles. It will no doubt have been noticed that the figures for the alkali reserve of whole blood or of corpuscles as discussed in the preceding pages, exhibit

an irregularity in relation to other determinations made on the same blood, so that all the relations of the alkali reserve of the corpuscles are obscured. The explanation is, I think, to be found in the fact, mentioned by Zuntz (*l.c.* p. 82) and since repeatedly confirmed, that the carbon dioxide fixation by blood is diminished on standing. Christiansen, Douglas and Haldane (*l.c.*) found that the power of fixing CO_2 was reduced by defibrination, and a similar phenomenon has recently been investigated by Mellanby and Thomas[20], who attribute it to a production of lactic acid by the corpuscles, which would, in the terminology of Haggard and Henderson (*l.c.*), increase the acid load of the corpuscles. The last named workers have also investigated the conditions under which the CO_2 fixation of shed blood is reduced, and find that the effect is more marked with defibrinated than with oxalated blood, but occurs in both, provided that the blood is exposed to a low carbon dioxide pressure.

Some of the results of my own experiments on this phenomenon are given below (Exps. 9–11). In addition to confirming what is already known regarding this fall in alkali reserve of the blood on standing, these experiments bring out the following points:

(1) The appearance of the effect is irregular (sometimes absent).

(2) The effect is accelerated by rise of temperature but is not completely stopped by a temperature of 0° C. (Exp. 10).

(3) The phenomenon affects the CO_2 carrying power of the corpuscles more than that of the plasma, which is often quite unaltered when the blood is again brought to alveolar CO_2 pressure, after having been kept at lower CO_2 pressures for considerable periods (Exps. 9, 10). (I have, however, met with cases in which, when the total change was small, the alkali reserve of plasma was reduced more than that of the corpuscles.) It follows from this, that the H-ion concentration of a blood, depending on the alkali reserve of the plasma, is likely to be less altered by keeping than is the alkali reserve of the whole blood—a very important fact, and one which I believe may explain in a satisfactory manner the very apparent discrepancies between the alkali reserves and reactions of bloods which have been kept for varying lengths of time. The fact that the change in alkali reserve affects the corpuscles more than the plasma is no doubt explained by the occurrence of the ionic interchange as soon as the plasma alkali tends to fall. The plasma alkali is thus kept up at the expense of the corpuscles which steadily absorb HCl.

The plasma does not entirely escape from the effects of preservation of the blood, and the result is that, on keeping, especially at body

temperature, the reaction of the blood slowly becomes somewhat more acid. (This is commented on by Michaelis, in his description of the hydrogen electrode technique, and I have myself observed it in hydrogen electrode experiments.) The effect on the reaction of blood of allowing it to stand is shown in Exps. 9 and 10. Exp. 9 shows that while the *p*.H of the blood at 45 mm. is unaffected by the fall in alkali reserve of the corpuscles, the reaction at 10 mm. is appreciably more acid in the old blood of low alkali reserve. Exp. 11 shows the effect of heating blood to 48°; below 70 mm. CO_2 the heated blood is more acid than the fresh blood.

Exp. 9. Human blood oxalated and kept standing in air at 20°, samples being withdrawn at intervals.

Time after drawing	*p*.H at 38° C.		Alkali reserve of		
	at 10 mm.	at 45 mm.	blood	plasma	corpuscles
10 mins.	—	7·53	—	—	—
30 „	8·0	—	60	64	52
1·5 hrs.	—	7·52	—	—	—
3·5 „	7·88	—	—	—	—
4 „	—	7·52	—	—	—
4·5 „	7·84	—	—	—	—
6 „	—	—	53	64	31

Exp. 10. Dog's blood, oxalated.

Blood kept for	Kept at 20°			Kept at 38°	
	alkali reserve blood	alkali reserve plasma	*p*.H at 20°, and 45 mm.	alkali reserve blood, at 20°	*p*.H at 38° and 45 mm.
30 mins.	—	—	7·14	—	—
1 hr.	54	—	—	—	7·28
1·5 hrs.	49	—	—	41	—
3·3 „	—	45	7·16	—	—
4·8 „	—	—	7·15	—	—
6 „	—	—	7·14	—	7·22
7 „	43	—	—	39	—
24 „ (of which 18 at 0° C.)	41	44	7·15	39	—

Exp. 11. Fresh oxalated goat's blood had an alkali reserve of 56 p.c.: its R-CO_2 curve was determined as rapidly as possible at 38°. Meanwhile some of the same blood was rotated in a saturator for one hour at 48° at 10 mm. CO_2, after which its R-CO_2 curve was plotted; the alkali reserve after the hour was 52 p.c. The reaction figures derived from the curves by interpolation were as follows:

	CO_2 pressures. mm.							
	20	30	40	50	60	70	80	90
p.H fresh blood	7·70	7·58	7·48	7·40	7·34	7·27	7·24	7·19
p.H heated blood	7·61	7·50	7·43	7·36	7·31	7·27	7·24	7·21

The calculation of the hydrogen ion concentration of blood. In a previous section it has been shown that the Hasselbalch method, for calculating H-ion concentration, can be applied to plasmas, and, with some slight

modifications, may be adapted to give results which are in agreement with those obtained by the indicator method, just as the unmodified formula introduced by Hasselbalch gives results which agree with those obtained by the electrode method.

Attempts to calculate the reaction of the blood would appear to be almost hopeless. Yet, it has been made, and happily with some success, by Hasselbalch and more recently by Parsons(27), who tried to discover some general mathematical expression which would relate the reaction of the blood to the carbon dioxide pressure. Even the improved formula of Parsons, however, is only applicable over certain ranges of CO_2 pressure, as shown by Davies, Haldane and Kennaway(8), and this is an indication that there is some factor not allowed for. Both Hasselbalch and Parsons clearly recognised that the bound CO_2 of a given blood is subject to very wide variations (when compared with a bicarbonate solution) according to the pressure of carbon dioxide to which the blood is exposed; Hasselbalch takes this into account by actual determination, in each case, of the bound CO_2 at the required CO_2 pressure, while Parsons has attempted to reduce this also to a mathematical expression by introducing a provisional constant which expresses the share taken by the proteins of the blood in competing with carbon dioxide for the possession of the available base, the latter being determined once for all by independent methods. But although Parsons(26) has shown that the *p*.H of a blood as calculated by Hasselbalch's method is lower than that found experimentally, both Hasselbalch and himself subsequently have implicitly, if not explicitly, regarded blood as a homogeneous fluid of uniform composition, since neither observer appears clearly to have taken into account the fact that while the *reaction* of blood is that of its plasma, the *carbon dioxide fixation* is a property which is shared by both plasma and corpuscles—a difficulty which has been pointed out by Joffe and Poulton(19), who suggest the only possible remedy, that of separating the plasma at the required CO_2 pressure, and determining its content of bound CO_2.

The relation between the hæmoglobin and corpuscular content. In dealing with the relation between corpuscular content and reaction of the blood, I have written as though the corpuscles as such, rather than their hæmoglobin content, were responsible for the phenomenon of secondary buffering. This was on account of the absence of precise knowledge either the one way or the other. I have made several ineffectual attempts to decide the matter by comparing the R-CO_2 curves of whole and

hæmolysed blood; the results were indecisive since I never found it possible to prepare hæmolysed blood free from stromata without at the same time altering both its alkali reserve and its hæmoglobin content. Michaelis and Davidoff(22) find that laked blood is usually more acid than unlaked blood, and this has also been my own experience. But such experiments as I have carried out do not in my opinion warrant the conclusion that the corpuscles exert any effect as secondary buffers apart from that due to their hæmoglobin content, though like Milroy(24) I find that the reaction of laked corpuscles is more acid than that of blood under similar conditions. It is interesting to note in this connection that Rohonyi and Lóránt(30) find that the bicarbonate and chloride shift, as determined by analysis of the ultra filtrate, is the same in hæmolysed as in whole blood, and it may be provisionally concluded that the corpuscular envelope plays a relatively unimportant part in determining the buffering action of the corpuscles.

III. Relation between the reaction of blood and that of its plasma.

In the present paper I have started from the assumption that the reaction of a blood is that of its "true plasma" or "true serum," in the sense in which these terms are used by Joffe and Poulton(19). For the investigation of these relations between the blood and the plasma the R-CO_2 curve of a blood was plotted, and at certain CO_2 pressures specimens of the plasma were withdrawn by centrifugation without loss of carbon dioxide. The R-CO_2 curves of these separated plasmas were also determined; the results of four typical experiments are shown in Figs. 12–15; the separated plasma curves and that of the parent blood being drawn on the same coordinates. When the carbon dioxide pressure of the separated plasma is equal to that at which the plasma was separated from the blood, then the reaction of the separated plasma (*i.e.* of the "true plasma") is the same as that of the blood at that pressure, as the curves show. It is also seen that there is a tendency for the plasma curve to cut the blood curve more or less boldly at this point, *i.e.* for the reactions of blood and plasma to be different at all CO_2 pressures, other than that one at which the plasma was drawn off from the blood. This statement however needs some qualification, since it does not hold good for plasmas removed at all pressures of carbon dioxide, but only within certain limits. The exceptions are two in number: firstly when the plasma is drawn off at a carbon dioxide pressure below a certain minimum, the plasma and blood curves run together

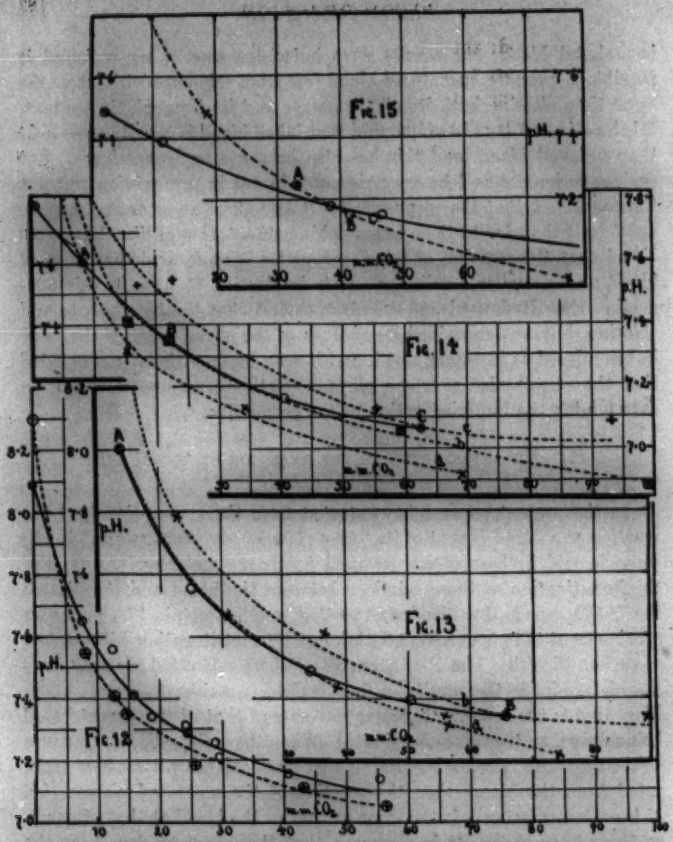


Fig. 12. R-CO_2 curves for cat's blood (A.R. = 53 p.c.) and of plasma drawn from it at 3·5 mm. of CO_2. The alkali reserve of the plasma = 34 p.c.

Fig. 13. Curves for horse blood (A.R. = 65 p.c.: corpuscular volume = 39·8 p.c.: oxygen capacity = 19·1 p.c.) and for plasmas separated from it. Plasma (*a*) was drawn off at point *A* = 3·8 mm.; plasma (*b*) at point *B* = 66 mm. The whole of the curve (*b*) is not shown. Alkali reserve plasma (*a*) = 53 p.c.; (*b*) = 85 p.c.

Fig. 14. Curves for blood of rabbit (corpuscular value = 32 p.c.) and for plasmas drawn off; (*a*) at *A* = 8·2 mm.; (*b*) at *B* = 22 mm.; (*c*) at *C* = 62·5. Alkali reserves: (*a*) = 35 p.c.; (*b*) = 47 p.c.; (*c*) = 57 p.c.

Fig. 15. Curves for blood of dog (alkali reserve = 42 p.c.: corpuscular volume = 54 p.c.) and for plasma drawn off at 38·5 mm. CO_2 (alkali reserve = 30 p.c.).

for some distance, to diverge ultimately as in the curve for plasma (*a*) of Fig. 13 (at 35 mm.): secondly, when the plasma is drawn above a certain limiting CO_2 pressure, as in (*b*) Fig. 13 and (*c*) Fig. 14, the blood and plasma curves join and run together at the higher pressures of CO_2, though at the lower pressures the plasma is more alkaline than the blood at equal CO_2 pressures. The minima and maxima at which the blood and plasma curves diverge from one another apparently differ considerably in different bloods. Their significance is clear. They mean that the secondary buffering by the corpuscles only takes place between certain limits of carbon dioxide pressure, say, in the horse blood (Fig. 13) between about 35 and 70 mm. CO_2; on either side of these limits, there is but little change in the bicarbonate concentration of the plasma with alteration of CO_2 pressure, because on the lower side of the minimum the secondary buffering has not yet begun, while on the part above the optimum, all the available base has been provided by the corpuscles, and thereafter the separated and true plasmas are identical. It will be remembered that a calculation made for horse blood (from another individual) in Exp. 5 showed that in all probability the blood corpuscles had reached or passed the limit of their buffering power (·06 m.) at the pressure of 85–90 mm., at which plasma (*b*) in Fig. 13 was actually drawn.

In a similar manner it can be calculated that the corpuscles of the rabbit's blood (Fig. 14) had at 62 mm. CO_2 yielded to the plasma an amount of alkali which would represent them as ·056 m. in base-yielding power at that pressure.

The variable manner in which the buffering appears in different bloods, together with the variation in their corpuscular contents, provides a satisfactory explanation of the wide differences between the bloods of different animals, and of the smaller variations between bloods of different individuals of the same species. Fig. 16 illustrates the way in which these differences emerge in the R-CO_2 curves for blood. The greater the corpuscular content the flatter the curve tends to become, *i.e.* the wider the range for secondary buffering. But even with bloods of nearly identical corpuscular contents the R-CO_2 curves exhibit very different degrees of steepness. In Fig. 16, a wide and intense buffering effect (often associated with high corpuscular content) will give such a flat curve as that represented by (A): this type is common in dog's blood, and is still seen even when the corpuscular content is fairly low. The curve cuts several plasma bicarbonate isopleths one after the other.

When the secondary buffering commences at a low CO_2 pressure and

finishes early, as in curve C, fewer isopleths are cut, *i.e.* after cutting a few, the curve runs with a bicarbonate isopleth, and is more curved than in the first example: cat's, goat's and rabbit's blood commonly conform to this pattern of curve. When secondary buffering is deferred until a measurable CO_2 pressure has been established, and is proportionately extended into the higher CO_2 portions of the graph, the curve (B) has a totally different appearance: this type is also met with in the cat, and in the horse. I have not examined a sufficient number of bloods to be able to say definitely that the ranges over which secondary buffering is effective are at all characteristic of certain species. From what has

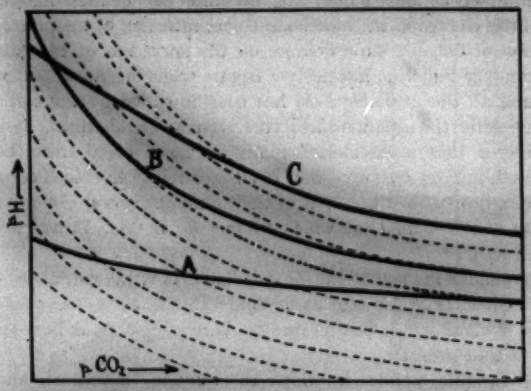


Fig. 16. Diagram showing three types of blood curve. The dotted lines are plasma bicarbonate isopleths. True plasmas separated at the points where these cut the blood curve would fall on these isopleths.

gone before, it would appear more than likely that it also depends on the acid load of the corpuscles, and so, on the previous history of the blood, both *in vivo* and in the laboratory.

It will be seen from Fig. 7 that the lower the alkali reserve of a blood the flatter the curve tends to be, *i.e.* the greater is the effect of the corpuscles in effecting the secondary buffering. This is because the relative amount of secondary buffering is greater when there is less bicarbonate in the plasma as may be seen from Fig. 4.

I have been much impressed by the flat type of curve shown by the dog, as contrasted with the curved type shown by the cat, and have no doubt that it has an important bearing on the physiology of the

species. Dog's blood and rabbit's blood are both better buffered than cat's blood, and the differences may be related to the fact that the dog and rabbit, as hunter and hunted, both need provision for prolonged and violent physical exertion, whereas the cat belongs to a tribe which characteristically obtain their prey by stalking and pouncing.

IV. The influence of temperature on the reaction of blood.

It has been shown by Hasselbalch (*l.c.*) that the *p*.H of a bicarbonate solution at 38° is ·12 higher than that at 18°, and this, he points out, is the resultant of two opposed changes, the one a diminution in the amount of carbon dioxide dissolved at the higher temperature, and the other an increase in the degree of ionisation of carbonic acid. It would be reasonable to suppose that plasma undergoes a similar alteration in hydrogen ion concentration with rise of temperature, and this I have found to be the case. My results indicate, in fact, a somewhat greater difference between the *p*.H of plasma at two temperatures differing by 20° than was found by Hasselbalch for $NaHCO_3$, but it is a difference in the same direction as he found. Michaelis and Davidoff (*l.c.*), however, state that for a rise of 20°, the *p*.H both of blood and of plasma *falls* by ·21. This statement needs qualification, because they did not work with fluids in equilibrium with identical carbon dioxide pressures; their procedure was to determine the reaction at room temperature and then to transfer the electrode to a thermostat and re-determine the *p*.H when a constant E.M.F. had been reached. They worked then, with (roughly) constant concentrations of carbon dioxide, and not with carbon dioxide at constant tension. By a somewhat similar method Michaelis and Rona[23] concluded that a bicarbonate CO_2 solution had the same reaction at 38° as at 18°, a conclusion which has already been criticised by Hasselbalch.

It might be expected, from what has gone before regarding the acidification of kept blood, that the effect of a rise of temperature on the blood might be of an irregular nature, and this expectation is, on the whole, justified. It can be definitely stated, however, that although the magnitude of the difference is liable to modification, the direction is always the same, viz. the reaction of blood at a higher temperature is more alkaline than at a lower when both are exposed to the same pressure of CO_2. Two experiments are given in Table V in illustration of this. The *p*.H at 38° is ·09 higher than that at 19° in the first example (horse), while in the second instance the difference is 0·2 (human). This

TABLE V. *Effect of temperature on reaction of blood.*

Exp. 12. Horse blood. Alkali reserve = 55 p.c.

	$p.CO_2$ mm.					
	9·1	—	47·2	54·2	77·4	
Temp. 38° $p.H$ =	7·75	—	7·37	7·35	7·21	
	6·2	19·1	26·5	34·3	62	101
Temp. 19° $p.H$ =	7·97	7·52	7·45	7·41	7·2	7·01

Interpolated points.

	$p.CO_2$						
	20	30	40	50	60	70	80
$p.H$ at 38°	7·58	7·49	7·42	7·35	7·29	7·23	7·18
$p.H$ at 19°	7·51	7·40	7·33	7·26	7·20	7·14	7·10
Diff. =	·07	·09	·09	·09	·09	·09	·08

Exp. 13. Human blood. Alkali reserve = 60 – 53 p.c.

Interpolated points.

	$p.CO_2$ mm.			
	20	40	60	80
$p.H$ at 38°	7·78	7·55	7·39	7·26
$p.H$ at 20°	7·60	7·34	7·19	7·06
Diff. =	·18	·21	·20	·20

discrepancy is, I think, due to unequal alteration in the two bloods on standing.

SUMMARY AND CONCLUSIONS.

1. Determinations of the reaction of blood and plasma by the dialysis method give results of the same order as those obtained by the use of the hydrogen electrode; but the absolute values of the $c.H$ is different since there is a constant error in the determination by the latter method.

2. Although the view that the reaction of plasma, like that of a bicarbonate solution, is dependent on the ratio $\frac{[NaHCO_3]}{[CO_2]}$ is confirmed, it is shown that the actual reactions of a plasma and of a bicarbonate solution, of identical bicarbonate content, are not identical; the plasma is $p.H$ 0·3 more acid than the bicarbonate solution at the same CO_2 pressure. This difference is principally due to the sodium chloride present in the plasma.

3. The influence of the proteins of plasma is very small. There seems to be a very slight effect, similar to that of NaCl in lowering the $p.H$ of the solution, but there is no evidence of any buffering action by the proteins.

4. Plasmas from different bloods and of different alkali reserves have CO_2 reaction curves parallel to one another, the $c.H$ at a given

CO_2 pressure ascending in the same ratio as the $NaHCO_3$ is reduced (*i.e.*, *p*.H varies directly as log. alkali reserve).

5. A slight modification is suggested in Hasselbalch's formula, and new values are given for $p.K_1$, derived from observations on actual plasma by the indicator method, enabling results, identical with those obtained by the indicator method, to be calculated from the alkali reserve and the pressure of CO_2.

6. The carbon dioxide reaction curves for different bloods are not usually parallel with one another. The differences are due to differences of hæmoglobin content, to differences in the extent and range of the ionic interchange between corpuscles and plasma (secondary buffering), and to somewhat capricious chemical alterations in the corpuscles on standing, whereby the secondary buffering is liable to modification.

7. The greater the hæmoglobin content (and the lower the alkali reserve of the whole blood) the flatter the R-CO_2 curve of the blood tends to become.

8. The alkali generating capacity of blood corpuscles (about ·06 m.) is available over different ranges of *p*.H in different bloods. It would appear that the hæmoglobin molecule is capable of exerting more than one (maximum three) base-fixing valency.

9. Although the CO_2-fixing capacity of blood falls off on standing, it is the corpuscles which, at a given pressure of CO_2, are most affected; this is again on account of the important secondary buffering action by means of which change in the *p*.H of the plasma is corrected.

10. Calculations of the hydrogen ion concentration of whole blood from the carbon dioxide content and pressure cannot be accepted without reserve, because the former bears no exact relation either to the latter, or to the alkali reserve of the plasma.

11. The reaction of a blood is that of its "true plasma" (Joffe and Poulton). Owing to the secondary buffering, the reaction of the "true plasma" alters less rapidly than that of separated plasma when the CO_2 pressure is altered.

12. Plasma and blood, like bicarbonate solutions, are, at the same CO_2 pressure, more alkaline at higher than at lower temperatures, owing to the reduced solubility of carbon dioxide at higher temperatures.

Some of the apparatus used in these experiments was purchased out of a grant by the Government Grants Committee of the Royal Society.

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THE RECOVERY PROCESS OF EXCITABLE TISSUES.

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(*From the Physiological Laboratory, Cambridge.*)

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I. The relation between the decline of the electric response and the return of excitability.

In a previous communication on the nature of the recovery process(1) I pointed out that we knew very little about the relation between the subsidence of the electric response in a tissue and the recovery of excitability. The time relations of both these processes have been measured by various observers in nerve, skeletal and cardiac muscle, but the two measurements have not been made on one and the same preparation under the same conditions. The result has been that the exact relation between the two processes is not known although there has been a general tendency to regard the decline of the electric response and the return of excitability as different aspects of the same recovery process.

The suggestion that the two processes occur simultaneously was made in the first instance by Tait(2), who regarded the absolute refractory period as coinciding with the rising phase of the electric response and the relative refractory period as coinciding with its decline. Keith Lucas criticised this view(3), but on the other hand Mines(4) found that in heart muscle the duration of the electric response and of the refractory state were very closely related and Sulze(5) showed that in

the olfactory nerve of the pike the absolute refractory period coincided with the rising phase of the electric response. It seems unlikely, however, that Tait's hypothesis can be universally true, for it has been known for some time that in cardiac muscle at low temperatures or under the influence of certain drugs the absolute refractory state may persist for some time after the electric response has subsided.

Theoretically it should be a simple matter to discover what relation exists between the decline of the action current and the return of excitability, for it is only necessary to make consecutive measurements of these two processes in the different excitable tissues. In practice the greatest source of error will arise in measuring the time relations of the electric response. In medullated nerve the whole electric change may be over in a few thousandths of a second and unless the recording instrument can follow so rapid a change the investigation will be of little use. For this reason some preliminary experiments were made to see what degree of accuracy could be expected from the electrical recording instruments.

A. *Magnitude of error in measurements of the electric response.*

All the measurements of electric responses described in this paper were made with the capillary electrometer designed by Keith Lucas(6). The capillary tube had a diameter at the working part of ·035 mm. and a pressure of 23 cm. of mercury was needed to force the meniscus to this region. The photographic plate could be made to travel at any speed up to 87 cm. per second, the velocity being recorded by a tuning fork making 200 double vibrations a second. The records were analysed according to Burch's empirical formula by the machine designed by Keith Lucas(7) and calibration curves (giving the constants of Burch's equation) were made whenever the resistance in the electrometer circuit was altered.

The errors which are likely to arise will depend in part on the difficulty of making the measurements on which the analysis is based, and in part on the use of Burch's formula for the analysis. This formula was derived empirically and has been shown to hold over a wide range, but Hermann and Gildemeister(8) have pointed out that the results it gives cannot be more than a fairly close approximation to the true values of the electromotive force.

The most convenient method of measuring the total error would be to produce a change of potential of known time relations as like as possible to the response of a nerve or muscle and to see how far the

analysed electrometer record of this change agreed with its true form. The discharge of a condenser through a known resistance gives a change of potential which is not unlike the monophasic response of a nerve or muscle; its duration can be varied over wide limits by altering the capacity and the resistance in the circuit, and the time relations of the discharge are easily calculated. For this reason the measurement of known condenser discharges seemed to offer a simple method of testing the accuracy which could be expected from the electrometer records. A set of condensers giving capacities varying from ·005 to 1·0 mf. was arranged to discharge through a non-inductive resistance or through a damp thread connecting two non-polarisable electrodes. The condenser was charged to ·03 volt (a value slightly less than the potential difference produced by a muscle and slightly greater than that of a nerve) and discharged at the moment when the photographic plate was passing across the field of the electrometer. The terminals of the electrometer were connected to opposite ends of the resistance so that, on the discharge of the condenser, the potential difference would rise suddenly to ·03 volt and would then fall off exponentially in accordance with the equation

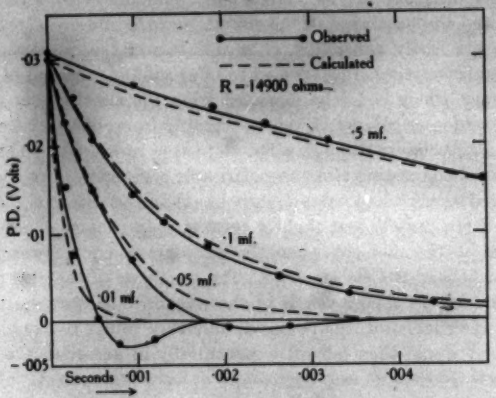
$$\log_e \frac{E_0}{E_t} = \frac{t}{R \times C \times 10^{-6}}$$

where R is the resistance in the circuit in ohms, C the capacity of the condenser in microfarads, E_0 the initial potential and E_t the potential after a time t seconds. Records were made with resistances of 1230, 10,000 or 14,900 ohms in the circuit and Fig. 1 shows the observed and calculated values for a number of discharges of widely different duration. It will be seen that in the most rapid discharges the observed potential overshoots the zero line. This might be due to a lack of damping in the condenser circuit; the dotted curve is calculated on the assumption that the self-induction in the circuit is negligible in comparison with the resistance and capacity and when the capacity is very small this assumption may not be strictly true[1]. It might also be due to a lack of damping in the electrometer, and this seems the more likely explanation as the overthrow was greater when a capillary tube of larger diameter was used. In any case there is bound to be a considerable error in the analysis of a discharge which is over in less than one thousandth of a second, for the recording surface only travels ·8 mm. in this interval. In the slower discharges, however, the agreement between calculated and observed values is surprisingly close and except at high temperatures

[1] The self-induction was not measured but it was probably not greater than a few microhenries and this would not be enough to produce an oscillating discharge.

the response of the frog's sciatic is slow enough to be within the range of fairly accurate working. This may be seen from Fig. 2, which gives

Fig. 1



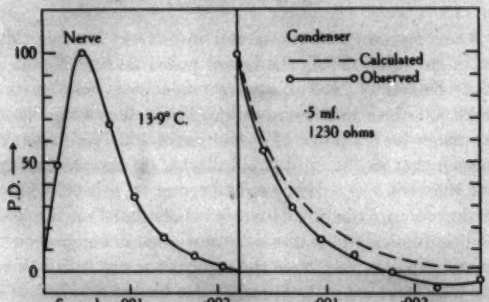


Fig. 2

Fig. 1. Discharge of condensers of different capacities. Comparison of calculated results with those observed by analysis of capillary electrometer records.

Fig. 2. Comparison of monophasic nerve response with condenser discharge to show probable error in records.

the analysed curves of a monophasic response of the frog's sciatic at 13·9° C. and the discharge of a condenser of ·5 mf. capacity through a resistance of 1230 ohms, both curves being reduced to the same scale.

The rate of fall of the potential is almost the same in the two cases and as the discrepancy between observed and calculated results is very small in the case of the condenser discharge, we may safely assume that the record of the nerve response gives a fairly accurate rendering of the actual change of potential in the nerve.

Fig. 3 shows the photographic records of monophasic muscle and nerve responses and of two condenser discharges of different capacity. In all 26 records of condenser discharges were made: in four of these the whole discharge occupied less than ·001 sec. and the difficulty of

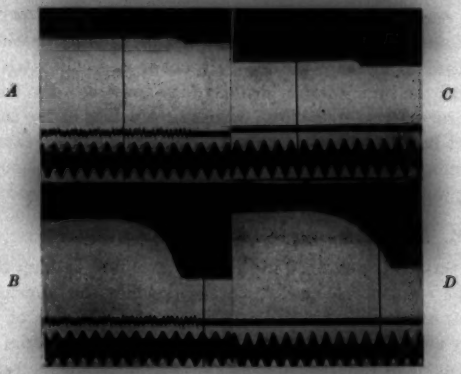


Fig. 3. Typical electrometer records before analysis, reduced to two-thirds of original size. A, Monophasic nerve response at 5·5° C. B, Monophasic response of sartorius at 15° C. C, Record of condenser discharge; capacity ·15 mf. Resistance 10,000 ohms, initial E.M.F. ·25 volt. D, Record of condenser discharge; capacity 1·5 mf. Resistance 10,000 ohms, initial E.M.F. ·3 volt. Tuning fork makes 200 D.V. per sec.

analysing the record made the final result untrustworthy, but of the remaining 22 where the duration was longer, only two showed any serious distortion of the curve and this was due to the appearance of a "sticky" region in the tube. The probable error in the case of nerve responses must be regarded as slightly larger than that shown in Fig. 1 since the maximum potential difference for a nerve response was usually nearer ·015 volt and the resistance in the circuit was larger. Both these differences would tend to reduce the excursion of the mercury in the capillary and the record of the excursion would become somewhat more difficult to analyse, but the increase in the error would be very

small and in two records made with condensers charged to ·01 volt the agreement was quite as good as that in Fig. 1.

It appears then that the capillary electrometer, corrected by Burch's method, gives a reliable means of timing the very rapid changes of potential which occur in a nerve response. An uncorrected string galvanometer record would show a much greater error, even though the string were under considerable tension, and although several methods have been proposed for the correction of string galvanometer records they are all far more laborious than the mechanical analysis of electrometer records by Lucas's machine.

As the research involves the time relations of the recovery curve as well as those of the electric response, the pendulum contact breaker which is used to measure the recovery curve was calibrated by comparison with the tuning fork which gave the time record in the electrometer measurements. As a check on the time values of the tuning fork the contact breaker was also calibrated more roughly by causing it to send currents of different durations into a ballistic galvanometer and comparing the deflections produced with those due to a steady current.

B. *Electric response and recovery in medullated nerve.*

The experiments described in this section consist in determining the form of the electric response which accompanies the propagated disturbance in a nerve fibre and comparing it with the rate of return of excitability after the passage of the disturbance. The material used was the sciatic nerve of the frog (*R. temp.*) and the experiments were carried out both in the summer and the winter months. The sciatic-gastrocnemius preparation was set up so that the nerve rested on two non-polarisable electrodes of the Ag—AgCl—NaCl-type devised by Lapicque(9). The electrodes are shown at *A* and *B* in Fig. 4 *a*: they could be used for stimulating the nerve or for leading off the electric response. When they were used for the electric response the stimulus was sent in by a pair of platinum electrodes *C* nearer to the muscle. In order that the electrometer should give a true record of the change occurring under the electrode *A* it was important that there should be no change of potential at the other electrode *B*, in other words the response had to be monophasic and not diphasic. To secure this the nerve was damaged by crushing or by heat at a point midway between *A* and *B* so that the impulse would be extinguished at this point.

The temperature of the nerve was controlled by covering the nerve chamber with a small metal tank containing water and ice or warm

water. The nerve lay about 2 mm. below the bottom of the tank and the temperature could be measured approximately by a small mercury thermometer with its bulb just below the nerve. In the earlier experiments a more elaborate water-jacketed chamber was used, but it was abandoned later in favour of the simpler arrangement because in the latter the nerve remained completely accessible throughout the experiment and the electrodes etc. could be adjusted without dismantling the apparatus. The thermometer indicated a steady temperature after the tank had been in place for ten minutes and the time relations of the electric response or the recovery curve did not change appreciably after this interval had been allowed.

The record of a nerve response is often deformed by an effect which

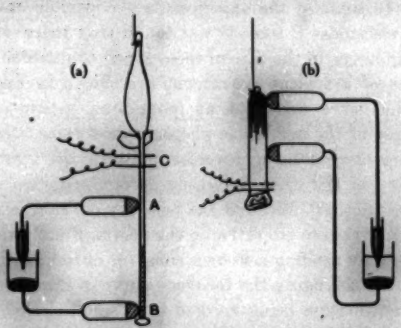


Fig. 4. Arrangement of electrodes for experiments on nerve and skeletal muscle. The direction of current flow through the electrometer circuit is immaterial.

is usually attributed to the spreading of the stimulating current into the electrometer circuit. In the nerve response shown in Fig. 2 the effect is just visible as a small upward movement of the mercury coinciding with the moment of stimulation. Forbes and Gregg(10) have shown that the effect is really due to the fact that the nerve has a considerable electric capacity, so that a current tends to flow from outlying parts of the nerve towards a point at low potential such as the kathodic electrode of the stimulating circuit. It can be reduced to negligible dimensions by placing the stimulating electrodes at some distance from the electrometer leads, by using a stimulating current which is not more than two or three times the threshold strength and by avoiding any connection between the nerve or electrometer and the

earth so as to make the capacity of the system as small as possible. As a rule the effect was only just visible in the records, but to guard against any error each record was usually repeated with the direction of the stimulating current reversed so that the effect would be of opposite sign in the two cases.

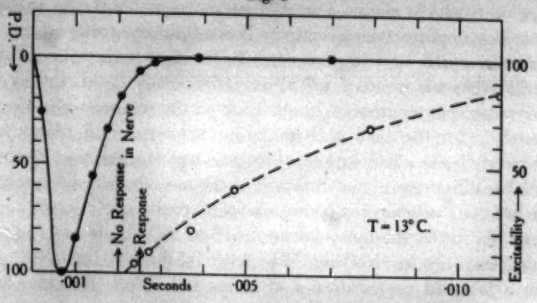
The return of excitability in the nerve was mapped out by sending in two stimuli at different intervals apart and finding the strength of the second stimulus needed to give a summated contraction in the muscle. The method has been described in detail in earlier papers. As we are concerned to know the rate of recovery at the point *A* where the electric response is measured, the stimuli were usually sent in through the two non-polarisable electrodes in such a direction that *A* would be the kathode. In some of the experiments the stimuli were sent in by the platinum electrodes *C* since it was found that there was never any appreciable difference in the rate of recovery at the two points.

The results of a typical experiment are shown in Fig. 5. In this experiment the nerve was kept at room temperature (13° C.). The electric response of the nerve was photographed twice, the direction of the stimulating current being reversed between the two records; the rate of recovery of the nerve was then determined, two more electric responses were photographed and the recovery curve was redetermined to detect any progressive alteration in the nerve. Finally the calibration curve was made by sending a known constant current into the electrometer circuit. In the figure the recovery curve is shown by the dotted line, each determination being marked by a small circle. The normal, or resting, excitability is taken as 100 and the excitability at different stages of recovery is expressed in percentages of the resting value. The monophasic electric response is shown by the full line, the corrected values of the potential difference being marked by small black discs. The response is inverted and the potential at any moment is expressed as a percentage of the maximum potential difference reached during the electric response. In this way the subsidence of the electric change is shown by a line rising from the base line (maximal potential) towards the line of zero potential and the recovery curve rises from zero excitability towards the same line, which in its case indicates the normal or resting excitability. This arrangement has been adopted in all the figures which compare the recovery curve with the electric response since it appears to give a clearer picture of the two events than any other method of presentation.

At first sight the figure seems to give a clear answer to the question

at issue. The absolute refractory period does not correspond to the period of increasing potential difference and the relative refractory period to its decline. Instead of this, the nerve remains completely refractory until almost the end of the whole electric variation, and the gradual rise of excitability is continued long after the electric change

Fig. 5



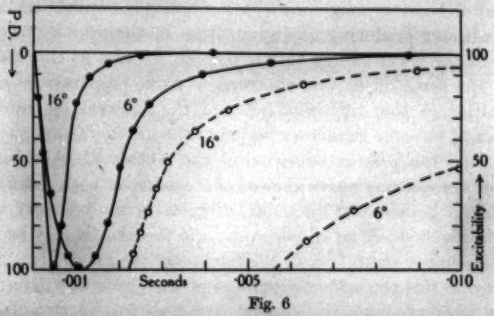


Fig. 6

Fig. 5. Monophasic electric response (full line) and return of excitability (dotted line) in frog's sciatic nerve.

Fig. 6. Electric response and recovery curve in frog's sciatic at different temperatures.

has subsided. If this evidence can be accepted it follows that Tait's suggestion cannot be true for medullated nerve. There are, however, one or two possible sources of error which need investigation before the result can be allowed to stand. In the first place the impulse which sets up the electric response is travelling away from the muscle towards the central end of the nerve. When the return of excitability is measured

we are dealing with an impulse which travels towards the muscle and not away from it. It is conceivable that the electric change accompanying a centripetal impulse might be of shorter duration than that accompanying a centrifugal, and if so the figure does not give a fair comparison between the electric change and the return of excitability. This possibility must be set aside for the following reasons. Bramwell and Lucas(11) have shown that the nerve recovers at the same rate whether the impulse which sets up the refractory state is travelling towards the muscle or away from it. To decide whether the time relations of the electric response are also unaffected by the direction of the impulse two experiments were made in which the response was measured alternately with the seat of stimulation proximal and distal to the electrometer leads. In neither of these experiments was there any appreciable difference in the duration of the electric response.

There is one other point on which some doubt might arise and this concerns the use of the muscular contraction as an index of the return of excitability. In his work on Wedensky inhibition, Lucas(12) found that in a fatigued preparation a stimulus falling on the nerve in the early stages of recovery might be able to set up a second impulse in the nerve without producing any summation in the muscle, owing to the failure of the nerve ending to transmit an impulse at this stage of recovery. The same effect was observed in fresh preparations cooled to 3° C., although the difference between the interval for muscular summation and the true refractory period of the nerve was not so great. An analysis by the present writer(13) of the factors which determine the shape of the recovery curve showed that whenever such a difference exists it should be revealed by a discontinuity in the recovery curve. The curve in Fig. 5 shows no discontinuity and therefore it may be taken as a true measure of the return of excitability in the nerve fibre, but to make certain of this the refractory period of the nerve was determined directly by photographing the electric responses to two stimuli. The result is shown in Fig. 5. A stimulus occurring ·0020 second after the first does not give a second electric response in the nerve, but one at ·0025 second does so. The least interval for muscular summation is ·0024 second, so that in this preparation at all events there is no doubt that the least interval for muscular summation agrees within narrow limits with the absolute refractory period of the nerve.

There is therefore no escape from the conclusion that the nerve remains inexcitable for some time after the electric response has begun to decline from its maximum value and that the whole electric change

is over long before the excitability has returned to its resting value. The result shown in Fig. 5 is typical of all the experiments carried out on nerve. Fifteen preparations were examined at temperatures ranging from 22° C. to 3° C. and in all of them the recovery of excitability appeared later and proceeded more slowly than the decline of the electric response.

Effects of varying temperature. To determine whether the relation between the two processes varies with temperature, the recovery curve and electric response were compared at two different temperatures in six experiments and at three in one exp. Fig. 6 shows the resulting curves in one of the former, the figure being constructed on the same lines as Fig. 5. The measurements were made first at 16° C. and then at 6° C. and it will be seen that the relative positions of the recovery curve and the electric response are very much the same at both temperatures. In either case the earliest return of excitability occurs just before the complete disappearance of the electric response although the least interval for summation is ·0057 sec. at 6° C. and only ·0023 sec. at 16° C.

Table I gives a series of values for the temperature coefficient of the

TABLE I.

Exp.	Temperature coefficient for 10°: Decline of electric response	Return of excitability	Range of temperature
1	4·05 2·5	4·55 2·35	6° and 13·5° C. 13·5° „ 21° C.
2	2·72	3·3	10·8° „ 23° C.
3	4·0	3·4	6·0° „ 16° C.
4	2·32	2·75	5·5° „ 16° C.
5	2·75	3·0	6·0° „ 16° C.
6	2·81	3·1	8·5° „ 15° C.
7	3·25	3·7	6·5° „ 16·5° C.
	Average 3·05	3·27	

declining phase of the electric response, measured by the time taken for the response to fall from its maximum to half its maximum value at different temperatures. The coefficient is calculated for a change of temperature of 10° C. The third column gives the temperature coefficient of the recovery of excitability for the same preparation. In this case the interval chosen is from the moment of stimulation until the moment at which the excitability has returned to half its resting value.

In spite of a considerable variation from one preparation to another there is a general agreement between the temperature coefficient of the two processes, although that of the return of excitability is slightly

higher than that of the decline of the electric response. The average value for the temperature coefficient works out at a little over three for a ten degree rise of temperature between 6° and 16° C. The time measurements are not accurate enough to give a satisfactory value for the temperature coefficient of the rising phase of the response, since the total duration of this is less than ·0005 sec. at the higher temperatures. The best records appear to show a coefficient which is nearer 2 than 3, but the result cannot be insisted upon.

We may conclude that the time relations of the electric response and of the return of excitability bear much the same relation to one another whatever the temperature. The recovery curve lags behind the decline of the electric response but the relation between the two is not altered by a change of temperature.

C. *Skeletal muscle.*

Similar experiments to those on medullated nerve were made on the sartorius muscle of the frog, stimulated directly. The sartorius was set up in the chamber designed by Keith Lucas(6) for working on the electric response of muscle. The arrangement of electrodes etc. is shown in Fig. 4 *b*. The tibial end of the muscle was connected to a light tension lever; the electrometer leads were arranged, one on the tibial end of the muscle and the other about midway between the tibial and pelvic ends. The stimulating electrodes were placed at the pelvic end so as to lie well outside the region of nerve endings. This precaution is necessary if the muscle is not curarised, since we have to determine the rate of return of excitability in the muscle fibres themselves. In many of the experiments the muscle was placed for an hour in ·03 p.c. curare before use. This strength would be quite enough to abolish all nerve or nerve ending effect and it is then possible to apply the stimulating electrodes at any point on the muscle without the risk of exciting nerve fibres, so that the recovery curve can be determined at the same point from which the electric response is led off. There is, however, but little difference in the rate of recovery at different points in a curarised muscle and as this agrees with the rate in an uncurarised muscle stimulated at the pelvic end, we may safely take the latter determination as giving a true value for muscle fibre.

As it was necessary to record the monophasic response of the muscle, the tibial end was damaged by burning with a hot iron so that the fibres became white and opaque for at least 3 mm. from the tip of the muscle. This damage reduced the strength of the contraction to about

two-thirds of its original value, but it seemed to have little immediate effect on the rate of recovery. Unfortunately the recovery curve of a muscle stimulated directly cannot be determined with anything like the accuracy possible in the case of a nerve. There is much greater difficulty in keeping a constant threshold and a constant height of

Fig. 7

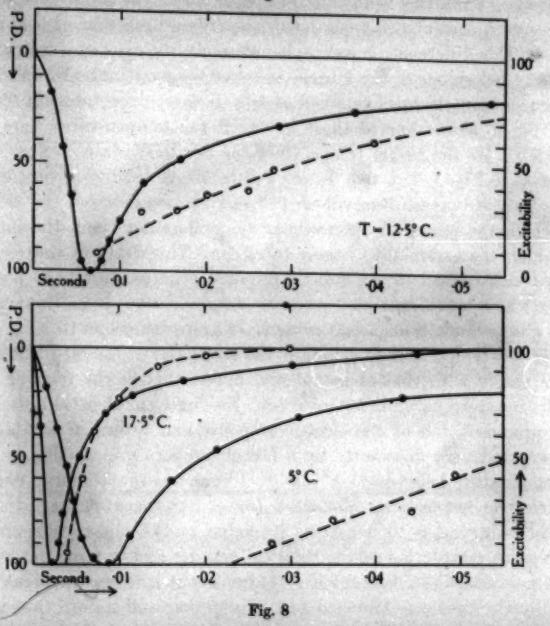


Fig. 8

Fig. 7. Monophasic electric response and recovery of excitability in frog's sartorius.
Fig. 8. Electric response and recovery curve of sartorius at different temperatures.

contraction in response to a single stimulus. This can be partially overcome by using weak stimuli which will not damage the more excitable fibres and by arranging the electrodes so that they will not be disturbed by the contractions of the muscle.

Five preliminary experiments were made at room temperature and all of them gave a very close agreement between the decline of the

electric response and the return of excitability. A typical record is shown in Fig. 7 which gives the two curves for a sartorius at 12·5° C.; the muscle was stimulated at its pelvic end and was not curarised. It will be seen that the absolute refractory period coincides more or less closely with the rising phase of the electric response and the return of excitability takes place during, and not after, the decline of the electric response. Thus the behaviour of the sartorius at room temperature agrees with that of non-medullated and differs from that of medullated nerve. This difference, however, is not so clearly marked if we consider also the behaviour of the muscle at lower temperatures. In six experiments the muscle was examined at two or three different temperatures and Fig. 8 shows one of these in which the temperatures were 17·5° and 5° C. At the higher temperature the two curves are in close agreement as in Fig. 7, but at 5° C. the return of excitability is very greatly retarded, so that an interval of ·016 sec. elapses between the moment at which the potential difference is at its maximum and the moment at which the excitability begins to return. This delay of the recovery curve with respect to the electric response occurred in all the preparations which were examined at temperatures below 7° C. (four in all) and was present, though less evident, in preparations at 10° C.

At low temperatures, therefore, the behaviour of the sartorius agrees more nearly with that of medullated nerve, though the relative delay of the recovery curve is not so great. Evidently in skeletal muscle the two processes, fall of the electric response and return of excitability, are affected very differently by a fall of temperature. In Fig. 8 a fall of temperature between 17·5° and 5° C. prolongs the declining phase of the electric response by about 3·2 times, but the absolute refractory period is increased to 5·6 times its value at the higher temperature. This great retardation of the recovery process at low temperatures has been pointed out by Bazett(14). He finds that it is most evident when the muscle has been damaged and it is probable therefore that in the present experiments the damage to the tibial end of the muscle may account for a part of the effect. Even in an undamaged muscle, however, the recovery is often greatly retarded at low temperatures for it was found in several experiments before the tibial end was burnt.

At high temperatures, *i.e.* over 20° C., for winter frogs, the first sign of recovery may appear just before the electric response has reached its maximum. An effect of this kind is shown in Fig. 9 where the muscle was at 24° C. The electric response was unusually slow in its development and it will be seen that the absolute refractory period is over

very slightly before the maximum potential difference is reached. Sulze found the same effect in some of his experiments on non-medullated nerve and suggested that it was due to the presence of a few fibres which recovered more rapidly than the majority. As the electric response which we measure is the sum of the responses in all the fibres, the time at which the total response is at its maximum will not necessarily coincide with the time of maximum response in the fibres which react most rapidly. It is difficult to test this suggestion experimentally but it would certainly account for the effect seen in Fig. 11.

Table II shows the temperature coefficients of the electric response and of the recovery of excitability in six preparations of the sartorius,

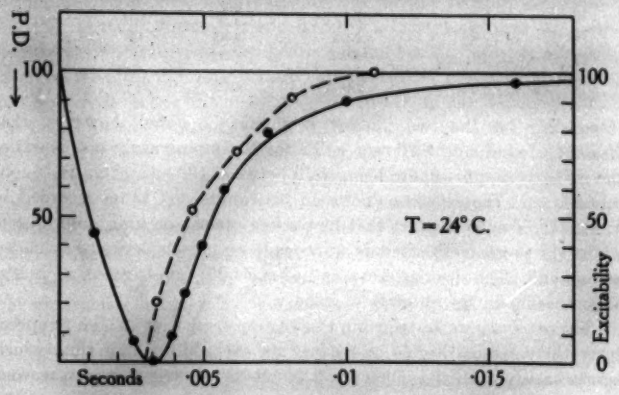


Fig. 9. Electric response and recovery curve of sartorius at 24° C.

and that of the electric response only in five more. The coefficients have the same significance as those in Table I. The variation from one temperature interval to another and from one preparation to another is much greater than in the experiments on nerve, but it is clear that the coefficient of the return of excitability is greater than that of the decline of the electric response. The average value for the latter is 2·9 (for nerve it was 3·05) whereas that of the recovery curve is 4·02. The temperature coefficients of the rising phase of the electric response are given in the second column; they give an average value of 2·66 which is distinctly lower than that of the declining phase. The difference is scarcely great enough to establish a distinction between the processes

TABLE II.

Temperature coefficient for 10°

Exp.	Electric response: Rising phase	Electric response: Declining phase	Return of excitability	Range of temperature
8	3·0	3·6	—	3·5° and 8° C.
	2·85	2·85	—	8° „ 15° C.
9	4·0	4·0	—	10° „ 17° C.
	2·2	2·4	—	17° „ 26° C.
10	2·15	3·55	—	5° „ 15° C.
	2·0	1·4	—	15° „ 25° C.
11	?	3·5	—	4° „ 14·5° C.
12	3·2	2·4	—	5·5° „ 17·3° C.
	3·06	3·0	—	17·3° „ 24° C.
13	2·2	2·3	2·6	13° „ 22·5° C.
14	3·0	3·2	5·5	5° „ 17·5° C.
15	2·1	3·1	3·7	3·5° „ 15·5° C.
16	2·5	3·05	4·2	10° „ 15° C.
	2·32	1·95	3·65	15° „ 25° C.
17	2·7	3·0	4·5	5° „ 17° C.
	Average 2·66	2·9	4·02	

responsible for the two phases; it should be noted, however, that Lucas(6) found a much lower value for the temperature coefficient of the rising phase, his figure being 1·79 between 18° and 8° C. It is conceivable that the difference between his results and those recorded in Table II is due to the fact that he worked mainly on uninjured muscle whilst the present experiments were made on muscle severely damaged at one end. The discrepancy was not specially investigated as it had little bearing on the question at issue.

The experiments described in this section show that at high temperatures the sartorius begins to recover its excitability at a time which agrees closely with the moment at which the electric response reaches its maximum value, the relative refractory period corresponding to the period of decline of the electric response. At low temperatures the return of excitability is delayed relatively to the electric response and the latter may have completely disappeared at a time when the excitability is still much below its normal value. Thus at high temperatures the sartorius agrees fairly well with Tait's hypothesis, but at low temperatures the recovery is delayed in much the same way as that of the frog's sciatic.

D. *Cardiac muscle.*

There is already a considerable amount of evidence to show that the relative refractory period in the heart may outlast the whole of the ventricular complex, the most complete account being due to Trendelenburg(15). But from the point of view of the present research this evidence

suffers from the fact that most of it relates to the diphasic electrocardiogram and that this gives very uncertain information as to the rate of decline of the electric change at any particular point in the heart muscle. If we wish to compare the return of excitability with the decline of the electric response it is clear that we must record the monophasic electrocardiogram instead of the diphasic, and unfortunately this introduces several complications into the experiment.

It is possible to obtain monophasic responses in the heart if the records are taken immediately after the tissue under one electrode has been damaged, but the injury current dwindles rapidly and in a few minutes the response shows a small diphasic effect. The rapid failure of the injury current is presumably due to the peculiar structure of cardiac muscle. Normally the cells form a syncytium, but after some part of the syncytium has been damaged the healthy tissue can separate itself off from the damaged area so that the further escape of electrolytes is prevented. When this occurs the electrode on the damaged area is no longer in free communication with the interior of the muscle cells and the response becomes diphasic again. The difficulty of securing monophasic responses over a long period would not matter if the time relations of the cardiac cycle remained the same before and after damage: we could then map out the recovery curve at our leisure and compare it with the electric response taken immediately after damage. But the destruction of tissue often causes large variations in the duration of the ventricular complex, so that the comparison would be of little value. For this reason the attempt to map out the full recovery curve was abandoned and the experiment was confined to the measurement of the absolute refractory period and the comparison of this with the duration of the monophasic response. Such measurements could be carried out during the few minutes in which the response remained truly monophasic.

The method of experiment was as follows: the frog's heart was exposed and the tip of the ventricle connected to a recording lever. Stimulating electrodes were placed on either side of the base of the ventricle and non-polarisable electrodes on the apex and on a point at the base midway between the stimulating electrodes. The heart was brought to a standstill by a Stannius ligature and the apex of the ventricle was then damaged over a considerable area by burning with a hot iron. As soon as possible after the damage the heart was stimulated at intervals of a minute or more by groups of two induction shocks sent in by a rotating contact breaker and separated by an interval

judged to correspond fairly closely with the absolute refractory period. The second stimulus was made four or five times the threshold value so that it would take effect in the earliest stages of recovery, the resulting electric responses were photographed and the records analysed in the usual way.

Fig. 10 shows four records taken from an experiment at 14·5° C. The moments of stimulation are marked by arrows. In the upper two figures the second stimulus occurs at a time when the electric response

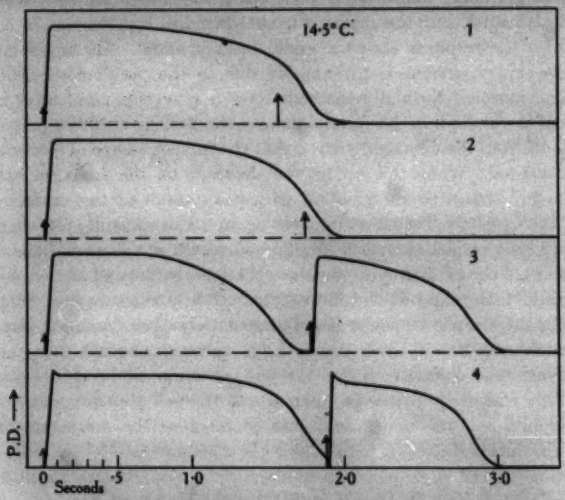


Fig. 10. Monophasic electric responses of frog's ventricle, showing duration of refractory period. Stimuli are marked by arrows.

has fallen considerably from its maximum value, but the tissue is still completely refractory and no second response is set up. In the lower two figures the second stimulus falls just after the end of the electric response, and a second response is set up, though its duration is shorter than that of the first response. The same result was found in four more experiments of the same kind. In all of these the decline of the electric response took place whilst the tissue was still completely inexcitable and the absolute refractory period was not over until just after the complete disappearance of the electric response. One experiment ap-

peared at first to contradict this result since a strong second stimulus produced a second electric response though it fell at a time when the first had not subsided. There can be little doubt, however, that this result was due to stimulation of the auricle, since the second response appeared after a very long delay and the effect disappeared completely when the stimulating electrodes were moved further away from the auricle. Trendelenburg states that a very strong stimulus may set up a second response before the first has subsided but in the present experiments this effect was not observed with stimuli five times the threshold strength.

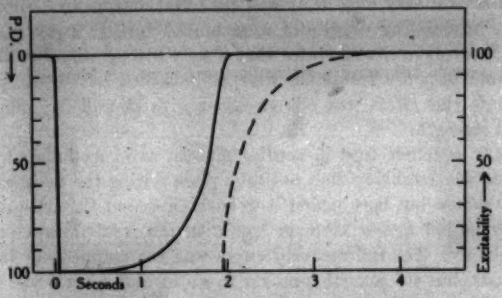
We may conclude that in cardiac muscle, as in medullated nerve, the return of excitability does not take place during the decline of the electric response but lags behind it to such an extent that the absolute refractory period agrees more or less with the total duration of the electric change. The full recovery curve was not mapped out in these experiments, but its general form for frog's cardiac muscle was shown in an earlier paper(1) and as we know the moment, in relation to the electric response, at which the return of excitability begins, it is possible to construct a figure showing the relation between the two processes by combining the record of a typical monophasic response with that of a typical recovery curve. Fig. 11 is constructed on these lines, the electric response being inverted as in the figures for nerve and muscle. If the great differences in the time scale are set aside it will be seen that the relation in cardiac muscle is very much like that in medullated nerve. It is worth nothing, however, that in cardiac muscle the return of excitability may be delayed even later than the end of the electric response. Kronecker and Stirling(16) found that in a heart at low temperature the refractory period might last into the middle of the diastolic pause, and Trendelenburg and de Boer(17) both find that after poisoning with certain drugs, *e.g.* veratrine and muscarine, the refractory period may last much longer than the electric response. Thus the relation varies to some extent with the temperature and condition of the muscle just as it does in the frog's sartorius.

E. *The form of the monophasic electrocardiogram.*

One other point in these records is of interest and this concerns the form of the monophasic variation in the frog's heart. It has been stated frequently during the past ten years that the electric response in the heart is made up of two components, a rapid phase (producing the *R* wave) due to the contraction of the fibrillar elements, and a slow

phase (producing the *T* wave) due to the sarcoplasm. It is admitted that the electrocardiogram in an undamaged heart is a diphasic change made up of the responses under the two electrodes, but it is held that

Fig. 11



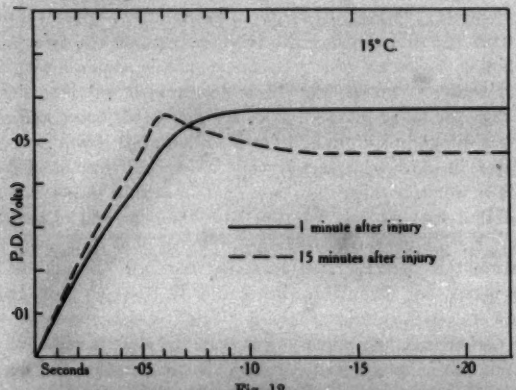


Fig. 12

Fig. 11. Probable relation between electric response and recovery curve in heart muscle. The curves are taken from two different preparations.

Fig. 12. Development of electric response in heart muscle, showing absence of initial spike in record taken one minute after injury.

the complicated form of the diphasic response cannot be explained without assuming that the response under each electrode is a double change made up of rapid and slow components. This theory, or some

modification of it, has been supported by Wertheim-Salomonson(18), Rümke(19), de Boer(20) and others and has been used as an argument that the twitch and the tonic contraction of skeletal muscle are due to the activities of different elements in the muscle fibre. The chief evidence in favour of this view is that in records of the monophasic electrocardiogram there is usually a distinct preliminary spike followed by a plateau at a slightly lower level. According to the more orthodox view such electrocardiograms are not truly monophasic and are due to the appearance of a small response in the damaged region. Such an explanation seems reasonable enough, for the injury current falls off very rapidly and as soon as it has begun to decline the electric response will cease to be truly monophasic. On the other hand very few records have been published in which the initial spike is not present although Samojloff(21) and Garten and Sulze(22) state that it does not appear in records taken immediately after injury.

The records taken in the present experiments are in complete agreement with the older view and opposed to that of a dual mechanism. If the response was photographed a minute or less after an extensive injury to the apex of the ventricle the curve showed no sign of an initial spike followed by a plateau. The curves marked 1, 2 and 3 in Fig. 10 were all taken within three minutes of injury and none of them gave any indication of a compound origin. The curve marked 4 shows the initial rise, but it was taken 4·5 minutes after injury and at that time the injury current had fallen to 88 p.c. of its initial value. There is ample reason, therefore, for the assumption that the response is no longer a pure monophasic effect. As all these records were photographed on a slowly moving surface they give little information as to the exact form of the rising part of the curve, and it is possible that a complex effect might be overlooked if it appeared before the potential difference had risen to its maximum. To guard against this, a series of responses were photographed on a rapidly moving plate; and two of the analysed curves are shown in Fig. 12. The full line shows the development of the electric response one minute after injury to the apex of the ventricle; the dotted curve shows the response 15 minutes later. The curve taken one minute after injury shows no sign of a complex origin; it rises steadily to its maximum and remains there. Fifteen minutes after injury the rise is somewhat more rapid (and the total duration of the response was shorter); the curve now rises to its maximum in ·062 sec. and then falls again, reaching a constant height at ·130 sec., *i.e.* in about twice the time it takes to rise. This is precisely the relation which might be

expected if the fall of potential is due to the appearance of a small response at the electrode on the apex, the rate of rise being the same at either electrode.

We may conclude that the form of the monophasic electrocardiogram gives no support to the theory that the heart muscle contains two contractile mechanisms of different time relations. The only important difference between the form of the response in cardiac muscle and that in skeletal muscle or medullated nerve lies in the fact that in cardiac muscle the rising phase of the response occupies a much smaller fraction of the whole response and the action current remains at its maximum value for a much greater fraction of the whole. The initial spike which is often observed on records which are thought to be monophasic is due, not to the activity of a separate, rapid mechanism, but to the appearance of a small diphasic effect as the damaged area is separated off from the healthy muscle[1].

II. The relation of the electric response to the period of supernormal recovery.

In an earlier paper (1) it was shown that if a tissue was perfused with an acid fluid, the recovery passed through a "supernormal period" before the normal resting condition was reached. During this period the excitability, conductivity and, in muscle, the contractile power are all greater than in the resting state. As the electric response in nerve may have subsided almost completely before the excitability has begun to return, it seemed very unlikely that the period of supernormal excitability could be associated with any peculiarity in the form of the electric response, but it was possible that some persistent after effect of the response had been overlooked and it seemed worth while to test the point directly. Two experiments were made on the frog's sciatic and seven on the frog's ventricular muscle. In the former, two sciatic gastrocnemius preparations were made from the same frog; the nerve of one preparation was bathed in Ringer of p. H 5·5 for one hour and that of the other in p. H 8·8. After this treatment the recovery curves showed the usual difference, that of the acid nerve giving a supernormal phase and that of the alkaline giving none. The monophasic responses of the two nerves showed no difference, however, and neither gave any sign of a continued positive or negative after effect. These experiments are scarcely conclusive because the electric response of a nerve is difficult

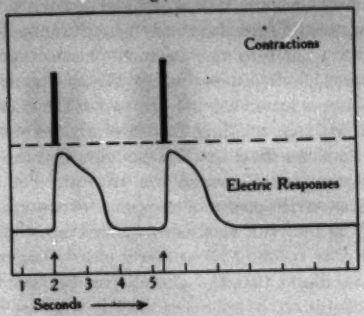
[1] Since the above was written Arbeiter has published a paper in which he comes to the same conclusion (*Arch. Néerl.* 5. p. 221. 1921).

to measure with great accuracy and the effect, if present, will probably be small. Cardiac muscle seemed to offer a better chance of detecting any effect which might be associated with the supernormal period and seven experiments were made on the following lines. The heart was perfused for half-an-hour with a fluid of known *p*.H and was then prevented from beating spontaneously by a Stannius ligature. The apex was burnt with a hot iron and the monophasic responses to groups of two stimuli were photographed whilst the contractions were recorded simultaneously on a smoked drum. In two of the experiments the heart was perfused with an alkaline fluid and the second contraction was never larger than the first; in the remaining five the fluid was slightly acid and the supernormal period was present. Fig. 13 shows, above, the contractions of the heart in response to two stimuli separated by 3·4 secs. and, below, the electric responses accompanying these two contractions. The second contraction is clearly larger than the first, so that there is no doubt that the second stimulus found the tissue in the supernormal condition, but the first electric response has subsided completely before the second stimulus occurs and there is no sign of any persistent after effect, positive or negative, since the potential falls to its original base line and stays there until the second response occurs. Several of the records do show a slight difference in the base line before and after an electric response, the meniscus never quite returning to its original position. But this change is merely an expression of the rapid decline of the injury current; it is associated with a return of the diphasic form of the electric response and it occurs equally in "acid" hearts which give the supernormal contraction and in "alkaline" hearts which do not. In none of the records was there any sign of a temporary difference of potential accompanying the supernormal period and disappearing with it.

It is of interest that the electric response which accompanies the increased contraction in the supernormal period is not itself greater than the first response. The same result was found in all the other experiments which showed a supernormal phase of recovery and it may be seen more clearly in Fig. 14, which records both the height of the contraction (measured from the point at which it diverges from the first contraction) and that of the *R* wave of the electric response occurring at different intervals after a previous response. The heart had been perfused with a fluid of *p*.H 6·5 before the Stannius ligature was applied and the electric response was led off from the uninjured ventricle. The height of the second electric response is given as a percentage of that

of the first response and the height of the second contraction as a percentage of that of the first contraction. It will be seen that the electric response is very little reduced even in the earliest stages of recovery

Fig. 13



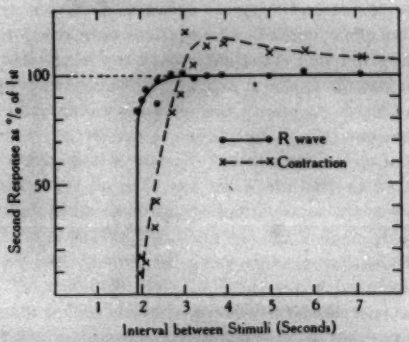


Fig. 14

Fig. 13. Electric response and contraction during supernormal phase of recovery.

Fig. 14. Size of electric response and contraction produced at different stages of recovery in heart perfused with an acid fluid.

and that it shows no signs of a supernormal rise although the second contraction is 113 p.c. as great as the first.

Two conclusions may be drawn from these experiments. In the first place the supernormal phase of recovery is not associated with a persistence of the electric response either as a positive or as a negative varia-

tion. In fact in the later stages of recovery the surface of cardiac muscle or nerve fibre is at the same electric potential as a surface which has regained its full excitability, and this result holds good whether the supernormal phase of recovery is present or not. In the second place the supernormal phase in the heart is not associated with any increase in the action current. Although the contraction is greater there is no sign of a greater output of the ions which produce the electric variation.

III. Discussion.

The chief outcome of these experiments is to show that the changes of excitability which follow the passage of an impulse are not necessarily associated with any difference of potential between the recovering region and a region at rest. In some tissues the return of excitability takes place during the decline of the electric response, but in others it does not occur until after the electric response has subsided. According to Sulze's results non-medullated nerve is the tissue which obeys Tait's hypothesis most exactly, for here recovery begins at the moment when the electric response begins to decline and this relation is true over a fairly wide range of temperature. Skeletal muscle comes next, for at high temperatures it obeys Tait's hypothesis, but at low temperatures the return of excitability is delayed until some time after the electric response has begun to decline. Medullated nerve agrees with non-medullated in that the relation between the electric response and the recovery curve is very little affected by a change of temperature, but the return of excitability does not begin until the electric response has subsided almost completely. Cardiac muscle comes last in the scale, for at room temperature the first return of excitability coincides with the end of the electric response and at low temperatures it appears that the absolute refractory state may last until some time after the electric response has subsided. It follows that we must abandon Tait's hypothesis that the relative refractory period coincides with the decline of the electric response, and with it we must abandon the idea that the diminished excitability of the relative refractory period is due to a residue of the action current which might interfere in some way with the effectiveness of the stimulus.

Before we can go further we must take some one theory of the conducted disturbance as a basis for discussion. The choice of one out of the many rival explanations of the nervous impulse sounds a formidable undertaking, were it not that there is one theory which stands alone in the precision with which it is formulated and in the number of facts

that it explains. This is the "membrane theory" put forward by Bernstein(23), Brünings(24) and Lillie(25), and recently developed by the last named by the aid of an amazingly close physical analogy(26). According to this theory the surface membrane of a resting nerve fibre is impermeable to certain anions which are present in greater concentration inside the fibre than outside it. The kations are free to pass through the membrane, but the electrostatic attraction of the anions will force the kations to remain close to the membrane so that an electrical double layer will be formed. The stimulating current (as in Nernst's theory of excitation) must produce a certain change in ionic concentration at the membrane, and at the kathode this will mean the removal of anions and, with them, the removal of the double layer on

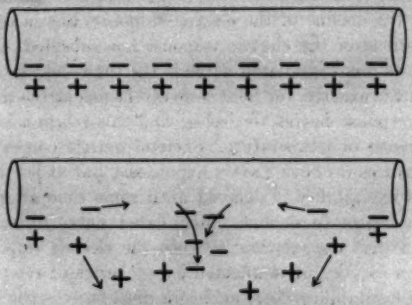


Fig. 15. Development of the electric response according to the membrane theory.

the membrane. This depolarisation is supposed to lead to an increase in the permeability of the surface at the kathode; the anions are now free to escape through the permeable area and their escape causes the action current, the corresponding kations being liberated from adjacent parts of the membrane as the anions move towards the permeable area. The action current leads to a depolarisation of the adjacent parts of the membrane which in their turn become permeable and the seat of an action current. Thus the change is propagated down the nerve fibre, the action current in one section forming the stimulus for the next section. The sequence of events may be seen more clearly from Fig. 15 which shows, above, the inactive fibre with the double layer at the surface, and, below, the active fibre with a permeable region through which the anions are passing out into the surrounding fluid. It will be

seen that the sections of the membrane adjacent to the permeable area have lost their double layer and, according to the main assumption of the theory, this will make them permeable in their turn and the change will extend along the whole fibre.

For the chief arguments in favour of the theory the papers of Lillie or Bernstein must be consulted. It has been shown to agree with the facts which are known concerning electrical stimulation, with the production of the action current and injury current, with the energy changes involved in the passage of an impulse etc. But in its simplest form the theory does not explain the existence of the refractory period and the changes of excitability which follow it. Once the membrane has been depolarised and lost its power of retaining anions there seems to be no reason why it should ever revert to the polarised, semipermeable condition again. To account for this at least two possible explanations might be put forward, and the present experiments seem to offer a means of deciding between them.

(1) *The exhaustion hypothesis.* The first suggestion is that the decline of the electric response is due to the exhaustion of the source of supply of the action current (*i.e.* the excess of ions inside the fibre) and that the return of excitability coincides with a slow restoration of the initial concentration by a balanced reaction which transforms an unionised precursor into an ionised substance. This leaves the change in the permeability of the membrane out of account, but in some respects the hypothesis is attractive were it not that the foregoing results show that the idea of exhaustion and replacement is untenable. If it were true we should find that a second stimulus could never lead to a further discharge of active material until the first response had come to an end and the tissue had had time in which to restore the concentration of ions inside it. But in the sartorius a second stimulus may be effective although it falls during the declining phase of the first response, and the action current may be made to increase again although there has been no period of complete inactivity during which the store of ions might be replenished. Since the return of excitability in the sartorius may coincide in time with the decline of the electric response it is clear that the one process cannot be due to an increase and the other to a decrease in the store of active material.

The behaviour of cardiac muscle and medullated nerve is more in keeping with the hypothesis as there is a greater delay in the return of excitability, but there is one further argument which applies to all tissues alike. Since the potential of an injured region is approximately

the same as that of an active region it is generally assumed that the immediate cause of the action current and the injury current is the same. On the membrane theory both are due to an increase in the permeability of the surface membrane and the consequent escape of anions. Now the injury current remains at or near its maximum value for at least several minutes after the injury. Consequently the store of ions is great enough, or is replenished rapidly enough, to account for a continuous flow of current for several minutes. Later on the injury current diminishes, but its decline is a very slow process compared with the decline of the action current. It is, of course, conceivable that the injury may upset the normal equilibrium between the "precursor" and the "active material," but Bernstein's evidence suggests very strongly that the injury current is maintained by processes occurring in the undamaged part of the fibre and that the injured region acts merely as an indifferent conducting path leading from the inside of the fibre to the fluid outside it[1]. So, if the normal fibre can maintain a supply of ions great enough to produce a continuous injury current for several minutes, it is very unlikely that an exhaustion of the supply can account for the failure of the action current after a few hundredths of a second.

Evidently we must abandon the view that the decline of the action current and the return of excitability is an expression of the exhaustion and subsequent replenishment of the store of ions which convey the current.

(2) *The permeability hypothesis.* An alternative view, if we accept the main outlines of the membrane theory, is that the action current is brought to an end because the membrane cannot remain permeable for more than a very brief space of time, since the action current itself causes a progressive decrease in the permeability of the active region. This suggestion has been worked out by Lillie(36). It involves the idea that the concentration of ions inside the fibre is maintained at a constant level above that outside and that the whole sequence of events from stimulus to recovery is determined by changes in the surface membrane of the fibre. The escape of anions from the active region serves not only to stimulate (*i.e.* render permeable) the adjacent inactive regions, but also to diminish the permeability of the surface through which the anions are passing. The membrane as it becomes impermeable again is at first much more stable than it was before activation, so that it is unaffected by the action current produced by the next section which

[1] But it should be pointed out that Pauli and Matula do not agree with Bernstein's conclusion (cf. *Arch. f. d. ges. Physiol.* 163. p. 355. 1916).

has now become permeable in its turn. Thus a circumscribed active region travels down the fibre causing the membrane in front of it to become permeable and rebuilding a more resistant, impermeable membrane behind it. A process of this kind is certainly not impossible, for Lillie has shown that it causes the wave of electro-chemical activity which passes down an iron wire immersed in strong nitric acid. According to this explanation the decline of the action current and the return to normal excitability are due to two distinct events, namely the reduction in the permeability of the membrane and the gradual return from a stable to a more unstable condition. These two events are no doubt closely related, but they would not necessarily occur simultaneously.

Now the chief conclusion which follows from the experiments in this paper is that the relation between the decline of the action current and the recovery curve varies considerably from one tissue to another and even from one temperature to another. Owing to this variation we have had to abandon Tait's hypothesis and the hypothesis of exhaustion, since both of these demand a fixed relation. But if the decline of the action current is due to one process and the return of excitability to another, we might well expect to find a variable lapse of time between the completion of the two events as we pass from tissue to tissue. For instance in skeletal muscle at room temperature we may suppose that the membrane recovers its normal instability rapidly so that it is always possible to reactivate it by a strong stimulus. In medullated nerve the membrane resists further change until it has returned to the impermeable condition and so the recovery curve lags behind the decline of the electric response.

Thus the present hypothesis will certainly account satisfactorily for the observed relation, or lack of relation, between the recovery curve and the electric response. There is, moreover, another line of evidence which gives it additional support. In the experiments on cardiac muscle it has been shown that an impulse set up during the supernormal phase of recovery does not produce an abnormally large action current. This must mean that the concentration of ions inside the fibre is no greater during the supernormal phase of recovery than it is when the fibre is at rest, so that the increased excitability and conductivity must be due to a greater instability of the surface membrane and not to an increase in the amount of "active material" in the fibre. This is clearly in agreement with the idea that the store of ions inside the fibre is maintained at a constant level and that the changes which account for activity and recovery are centred in the surface membrane.

But why should the passage of anions through the membrane in the active region cause it to become less permeable and at the same time more resistant to change than it was before activation? In general terms if we regard the active and inactive regions as the anode and kathode of the circuit produced by the action current it is likely that the current will produce opposite effects at the two poles, and as it makes the kathode region more permeable it should make that at the anode less. In Lillie's iron wire model the action current oxidises the surface of the active region and reduces that of the inactive; a thick film of oxide is deposited over the active surface and the subsequent thinning of this film accounts for the gradual passing away of the refractory state. As we know so little of the molecular structure of the nerve fibre we can only guess at the changes which may occur in the surface membrane during activity: the principal change may be an alteration in the thickness of the surface film, but it is at any rate worth considering whether the change may not be due to an interaction between the ions and the material which composes the membrane.

A suggestion of this kind has already been made in Part I of the present investigation in order to account for the appearance of the "supernormal phase" of recovery when a nerve is perfused with an acid fluid. It was there pointed out that a protein molecule or aggregate is generally most unstable in the isoelectric condition and that it becomes more stable if positively or negatively charged, *i.e.* combined with kations or anions. It was suggested that the surface membrane became negatively charged as a result of the action current and therefore relatively stable. The gradual loss of this charge accounted for the return of normal excitability, but in the presence of an excess of H ions the membrane passed through its isoelectric point and took on a small positive charge so that the excitability would rise to a maximum and would then fall again as the isoelectric state was passed.

Now the present experiments have shown that there may be no difference of potential between a recovering surface and one at rest, and this may seem to contradict the idea that the recovering surface can be negatively charged. But the notion of a charge as applied to the case of protein molecules in water means no more than the formation of a loose compound between the molecule and certain ions. A protein molecule may form a stable compound with either anions or kations and a surface composed of such molecules might be negatively charged (*i.e.* combined with anions) without showing any difference of potential towards a surface which was not so combined. During activity there

will certainly be an opportunity for the surface membrane to become combined with anions, for there is a continuous flow of these through the permeable area and the concentration of kations in the neighbourhood is reduced. On the analogy with a protein solution we should expect a compound so formed to be more stable and consequently less excitable than the resting membrane, and as the permeability decreases the normal distribution of electrolytes will return and the membrane will gradually revert to the uncombined condition where its excitability is at a maximum. The varying delay in the return of excitability in different tissues is easily explained on this hypothesis, for Mines has pointed out that the charges on different colloidal surfaces show varying degrees of inertia and may change rapidly or slowly in response to a new distribution of electrolytes (27).

All this does not account for the changes in the permeability of the membrane during activity, though it may explain the subsequent return of excitability. Possibly the mere combination of the membrane with anions makes it impermeable again or possibly a much more complex change takes place. In any case it seems that the "inertia" of the membrane is of fundamental importance. If the changes in permeability depend on changes in ionic concentration it is clear that the reaction cannot occur instantaneously or the electric response would never be allowed to develop at all. When it has once become permeable the membrane does not become impermeable again until some time after the anions have started to escape through it and the lapse of time necessary for the reaction to take place must vary enormously from one tissue to another.

According to this view the whole sequence of events from the stimulus to the completion of recovery would seem to depend on the rate at which the membrane responds to changes in the concentration of ions in its neighbourhood. In the nerve fibre the membrane must react very rapidly and all the processes concerned with stimulation, activity and recovery are over in a very short space of time: in cardiac muscle these events proceed at a much more leisurely pace because the surface membrane responds more sluggishly. Thus the different time relations of a "rapid" and a "slow" tissue may depend as much on the nature of the surface membrane as on the differences in the velocity of the ions which are demanded by Nernst's theory of excitation.

It would be useless to elaborate these views without further evidence. The membrane theory is in no sense a complete explanation of the conducted disturbance and even in its present form there are several

difficulties to be faced, notably the difficulty of bringing it into line with the phenomena of lactic acid metabolism during activity. But the experiments described in the present research show that the phenomena of recovery, the decline of the electric response and the return to normal excitability, can be made to harmonise with the membrane theory and in particular with that development of it which has been put forward by Lillie. To certain alternative theories they are definitely opposed.

Summary.

Experiments have been made to find out the relation between the decline of the electric response and the return of excitability in different tissues. The capillary electrometer was used to measure the electric response and its accuracy was determined by taking records of condenser discharges of short duration and comparing observed and calculated results. It was found that the analysed curves give a fairly accurate record of a change of potential as rapid as that of a nerve response.

The chief conclusions are as follows:

(1) In the frog's medullated nerve the absolute refractory period does not come to an end until the electric response has almost subsided. The gradual return of excitability (relative refractory period) takes place after the tissue has regained its normal potential. This holds good over a wide range of temperature.

(2) In the sartorius muscle the return of excitability comes on much more rapidly. At room temperature the absolute refractory period corresponds to the rising phase of the electric response and the relative refractory period to its decline. At low temperatures this relation is disturbed and the return of excitability takes place more slowly than the decline of the electric response.

(3) In cardiac muscle at room temperature the return of excitability does not begin until just before or just after the complete disappearance of the electric response and there is evidence to show that in abnormal conditions it may be still further delayed. Incidentally the monophasic electrocardiogram shows no signs of being made up of two components. An initial spike is only observed when the response has become diphasic.

(4) The supernormal phase of recovery which takes place in a tissue perfused with an acid solution is not associated with any prolongation of the electric response, either as a positive or negative after effect. An electric response set up during the supernormal phase of recovery in the heart is no greater than the normal although the accompanying contraction is increased.

(5) Thus the return of excitability does not necessarily occur at the same time as the decline of the electric response. The relation between the two processes varies from one tissue to another, and in muscle from one temperature to another. There is often no difference of potential between a surface which is recovering and one which is at rest.

(6) These results can be explained satisfactorily on the membrane theory by means of the scheme put forward by Lillie. According to this scheme the decline of the electric response is caused by a reduction in the permeability of the surface membrane and the return of excitability is due to a return of the membrane from a stable to a more unstable condition. The present work suggests that the change in stability may be due to the breaking down of a combination between the membrane and the anions to which it is exposed during the passage of the action current.

(7) There is no evidence that the refractory state is due to the exhaustion of the active material in the fibre. The excess of ions inside the fibre is maintained at a constant level and the events which occur during activity and recovery are due to changes in the surface membrane. The rate at which the membrane responds to changes in ionic concentration is of great importance in determining the rate of reaction of the tissue.

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THE PHYSIOLOGY OF THE VEINS. By J. F. DONEGAN

(1851 *Medical Research Exhibition, University College, Galway.*)

(*From the Physiological Institute, University College, London.*)

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THE occurrence of constriction or dilatation in the veins on nerve stimulation has received scant attention and the influence on the circulation of changes in venous calibre has received still less. Considerable nervous control of the portal vein, is, it is true, held to exist, but such control as has been said to exist over other veins has been regarded as of little importance in modifying the circulation of the blood. Direct experiments on the nervous control of veins have been made by Thompson (1893), Bancroft (1898), Ducchesci (1902), and Hooker (1918).

Thompson(1) on stimulation of the sciatic nerve saw band-like contractions of the ext. saphenous vein in rabbits and dogs, with the crural artery clipped or open. In only one case he saw similar contractions in the int. saphenous vein on stimulation of the crural nerve. He attempted to obtain further evidence by blocking the inf. vena cava below the kidneys and recording the pressure in the blocked off portion of the vein on stimulation of the cut spinal cord. His results were sometimes positive and sometimes negative. Bancroft(2) observed the same form of contraction in the ext. saphenous vein and traced the origin of the veno-motor fibres to the spinal cord. Ducchesci(3) clamped the artery and connected the central end of the int. saphenous vein with a manometer and obtained a rise of pressure on stimulation of the ant. crural nerve. In this case however the result may be partly due at least to constriction of other areas besides the veins. Hooker(4) stimulated the nerve accompanying the inferior mesenteric artery and obtained evidence by the manometer method of constriction of the inf. mesenteric vein. With stimulation of the vaso-motor centre directly by asphyxia and reflexly by stimulation of the central end of a sensory

nerve, he obtained the same results. Unfortunately, in his experiments, constriction of arterioles and capillaries cannot be excluded. It may be, as he says, possible that the constriction of the arterioles drives the fluid out through the artery, but if the artery end of the arterioles enters into contraction a shade before the other end, then the fluid cannot escape into the artery.

My experiments have been chiefly directed to comparing the effect of nerve stimulation on different veins, to determining the course and origin of the veno-motor nerves and the extent of central control and reflex effect.

Method. The experiments were chiefly carried out on cats, as the veins in these animals are more constant anatomically, and the tributary venules easier to tie than in either rabbit or dog. The results were, however, verified in the latter animals. The animals were anæsthetised with chloroform and ether. In a few experiments urethane was given intravenously, and where injection of curare was required to prevent muscular movements the animal was decerebrated. Three different methods were employed:

1. The vein to be tested was laid bare by dividing the skin over its course. The nerves to the part were dissected and stimulated and the results noted by inspection.

2. The vein was laid bare and all its tributaries tied. Its capillary end was tied, a cannula inserted into it and the blood washed out with Ringer's solution at 37° C. At a suitable point the central end was tied, a cannula tied in, and when the blood was washed out, the central cannula was attached to a water manometer. When the pressure on the manometer reached the usual venous pressure for the vein, about 60 mm. H_2O the inflow tube was clipped. The nerves to the part were stimulated, and the change in the water meniscus noted.

3. The procedure was similar to 2 except that the Ringer's solution from a reservoir kept at a temperature of 40° C. in a water bath was allowed to flow continuously through the tied off tube of vein. The rate of flow of the fluid was recorded on a revolving drum by an electric drop recorder.

In method 1 a diminution in the size of the vein might be ascribed to a constriction of the arterioles decreasing the amount of blood flowing into the vein but in 2 and 3 such effect was excluded. Method 2 was unsatisfactory, as blood kept continually trickling through minute pores in the wall—evidently the vaso-vasorum—with the result that the manometer level kept continually rising. In addition, to prevent

clotting, the outflow cannula had to be disconnected from the manometer, and the vein washed through with Ringer. By continual perfusion, this difficulty was obviated, but it had the disadvantage of sooner or later causing the death of the vein.

Most of the accessible veins have been studied, including the internal and external saphenous of the lower limb, the dorsal-radial of the upper limb, the external jugular, the vein of the large intestine, the superior mesenteric, and the inf. vena cava. The int. saphenous was the vein chiefly studied, owing to its easy access and its general suitability. A sketch of the vein is shown in (Fig. 1) the part x–y 10 to 12 cm. in length is admirably suited to the requirements of the experiment, as it can be tied with very little disturbance to the circulation of the limb since the tributaries joining it along this portion are all small and mostly from the skin. The ant. crural nerve, soon after its exit from the pelvis,

Fig. 1. Sketch of int. saphenous vein (right) of cat. V, vein. N, nerve. A, point where contraction begins. DM., deep femoral branch. B, branch to small or ext. saphenous vein.

gives off a branch which runs on the outer side of the vein to near the ankle. By cutting the muscular branches off the ant. crural nerve a nerve-vein preparation is obtained which can be removed from the limb if necessary, and studied in a warm bath of Ringer. Except while actually inspecting them, the veins were always covered with the skin flaps, while pads of cotton wool wrung out of hot saline were placed over the leg.

The effect of nerve stimulation.

Some initial experiments were carried out by method 2 on the int. saphenous vein. The ant. crural nerve was stimulated several times by induction currents. The stimulation time varied from 3 to 30 secs., and a weak current was used to diminish liability to fatigue of the venous nerve-endings. In all cases the slowly rising meniscus stopped dead, to begin its movement on cessation of the stimulus. This was due in all probability to the vaso-vasorum being shut off. In no case

did the manometer show the quick rise which would indicate contraction of the vein, neither did the vein visibly diminish in calibre.

The next experiments of the series were carried out by method 1. With weak current strength, the results were negative. I then tried very strong currents and in about 10–15 secs., the lumen of the int. saphenous vein had disappeared completely from its origin right up to the junction of the deep vessels. On stopping the stimulation, the vein returned after some minutes to nearly its original size. The secondary coil was then moved out step by step and the stimulation repeated. At a distance of 10 cm. where the current was scarcely perceptible on the tongue, the contraction was obtained. The vein and nerve on the opposite side were now dissected. The peripheral end of the cut nerve was stimulated with the secondary coil at 10 cm. but with no result, even after 60 secs. stimulation. The coil was moved up to 4 cm. when again a complete obliteration of the vein lumen occurred. The coil was then gradually moved out and at 12 cm. stimulation gave the same result. The result was obtained both with the femoral artery clamped and peripheral end of the vein tied, the latter excluding possible arteriole or capillary effect. In order to be certain the effect was not due to escape of current, the ant. crural nerve was stimulated in the abdominal cavity and the same series of phenomena was obtained. It seemed clear that the failure to cause contraction by method 1 mentioned above was due to the currents employed being too weak, and on repeating the experiments with strong currents a quick rise of the water meniscus gave evidence of the contraction of the vein. Further proof was obtained by method 3. Fig. 2 shows the great slowing of flow through the vein during the stimulation of the lumbar sympathetic cord.

As the vein is so closely accompanied along its whole course by the nerve, there remained a very remote possibility that the action of the current of the nerve, might be itself stimulating the muscle fibres of the vein. The connective-tissue sheath of the vein was therefore slit along its upper half, on the side remote from the nerve, and the vein very carefully separated from the sheath. It may be mentioned that the operation causes the vein to pass into tonic contraction which may last for 20 to 40 mins., due I think to the nerve filaments entering the vein, being mechanically stimulated. The operation if carefully conducted does not interfere with the close side-by-side position of the nerve and vein. On stimulating the nerve to such a "denervated" vein, when the latter has lost its tonic condition, it can be seen that the

denervated portion remains quite normal while the rest gives the usual constriction.

During some experiments on stimulation of the segments of the lumbar sympathetic cord to determinate the origin of the fibres a few facts were brought to light which may be related here. It was found in the majority of cases that stimulation between the 3rd and 4th lumbar ganglia was followed by localised contraction of the upper end of the vein, while stimulation between the 4th and 5th and 5th and 6th caused the whole vein to contract. But on stimulation of the segments with weak currents a marked difference showed itself in the reaction of the veins. From a series of 12 experiments, it became apparent that

Fig. 2 (a). Int. saph. vein (cat). B type, 3rd stimulation.

Fig. 2 (b). Int. saph. vein. A type, 1st stimulation.

In all figures, top line is drop second, middle line signal, bottom line, time in seconds.

the veins could be roughly divided into two classes—the numbers in them being about equal. (A) which reacted immediately to very weak stimuli, (B) which in most cases did not contract at all on the first stimulation of 60 seconds but reacted with a very long latent period and slow contraction on second stimulation with the same current strength. A comparison of the following two experiments, each being a fairly extreme example of its class will illustrate the point. The experiments were conducted in exactly the same way, and under conditions as nearly equal as possible.

The time measurements are rough, but they serve to show the points of contrast. In (A) there is little or no change in the duration of the

TABLE I. Quick type (A).

Cat. $CHCl_3$ and ether, intestines removed, lumbar sympathetic cord (L.S.C.) dissected on both sides. Both int. saphenous veins exposed, and the time noted before contraction appeared (L.P.), time required for contraction (C.T.), and time required for dilatation (D.T.), all in seconds. Stimulation of 4–5 L. segment with weak induction shocks.

	Right leg				Left leg		
Stimulations	L.P. secs.	C.T. secs.	D.T. secs.	Stimulations	L.P. secs.	C.T. secs.	D.T. secs.
1	5	13	90	1	6	15	80
2	5	10	90	2	4	15	85
3	5	12	100	3	5	12	90
4	6	10	90	4	5	13	80
5	6	11	90	5	6	14	90
6	5	12	100	6	4	12	90
			Two hours later				
1	6	12	90	1	5	13	90
2	6	10	105	2	7	12	100
3	6	13	100	3	6	14	100

TABLE II. Slow type (B).

Cat. Conditions exactly similar to those in Table I.

	Right vein				Left vein		
Stimulations	L.P. secs.	C.T. secs.	D.T. mins.	Stimulations	L.P. secs.	C.T. secs.	D.T. mins.
1	No result after 60 secs.			1	No result after 60 secs.		
2	50	60	10–12	2	45	65	11
3	40	55	8–10	3	35	50	8
4	20	50	6	4	20	40	6
5	15	50	6	5	15	35	6
6	15	35	6	6	15	35	6
7	15	40	5–6	7	15	40	5
8	12	40	5–6	8	15	40	6
			Two and a half hours later				
1	No result after 60 secs.			1	No result after 60 secs.		
2	50	70	12	2	40	70	11
3	35	50	8	3	30	55	7
4	20	35	5	4	18	40	5
5	20	40	5	5	20	40	6

different phases on successive stimulation. In (B), on the other hand, the duration of all phases continually reduces till a constant level is reached, usually after three to six stimulations, but even at this level the times are much greater than in (A).

The increased response resembles the facilitation process, which Sherrington(5) has shown to be of such importance in the central nervous system, and still more the phenomenon which Bayliss and Starling(6) have met with in the action of the vagus on the intestinal movements, and Anrep(7) in the action of the same nerve on pancreatic secretion. The type (B) vein returns to its normal condition in about

two hours and again shows facilitation on stimulation. Fig. 3 gives a rough idea of the effect.

In the veins of type (B) the course of the contraction can be readily followed. It usually—almost invariably—starts at point *A* (Fig. 1) quickly spreads down the dorsum pedis, and more slowly up towards the pelvic end, always—and this is common to both types—stopping short some distance peripheral to the entrance of the deep muscular veins. Owing to the point of origin of the contraction and to the presence of a valve near the middle of the crus, there is always distal to this valve a length of vein 1 to 2 cm. in a swollen condition. If this valve is absent, as sometimes happens, this swollen portion is also absent.

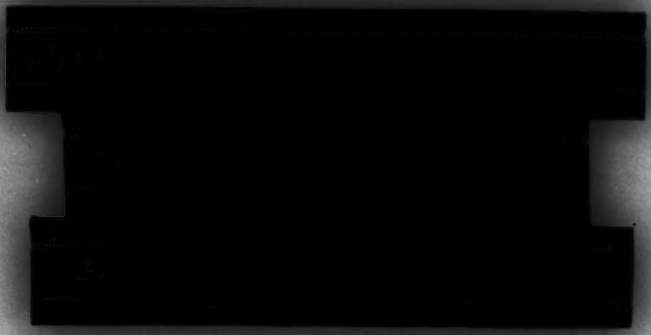

Fig. 3. Example of "facilitation." A, 1st stimulation of L.S.C.; B, 3rd; C, 5th.

The progress of the contraction is more difficult to follow in type (A) but is usually the same.

Very rarely has the band-like contraction described by Thompson and Bancroft been seen. It is exclusively met with in type (B) and it is probably due to the onset of contraction not being simultaneous over all parts of the vein. In this way it happens that segments of the vein wall contracting before distal adjacent portions, prevent the blood passing centrally the result being bulging pockets of blood with constricted bands between. After my attention was called by Prof. Elliot Smith to work on the innervation of the arteries of the human fore-limb (8), I found that by lifting carefully the nerve from the vein, small bundles of fibres could be seen passing from it to the vein at irregular intervals. In two cases of band-like contracting veins, it was plainly

seen that the bands of contraction were situated around the point of entrance of the nerve-bundles. The condition usually passes off after the first two or three stimulations, and the contraction becomes normal.

As to the cause of the marked difference in behaviour between types (A) and (B), I have not been able to find a satisfactory explanation, but a few observations on this point may be of interest. In the veins of type (B) it was apparent on exposing the vein, that it was flabby, its lumen ellipse-shaped, while in type (A) it was usually tonic looking and circular. This difference was so marked that one could almost always judge correctly what type it would turn out to be. With the first stimulation in type (B) usually no contraction occurred, but the vein became tonic looking and circular. On the next stimulation it contracted. When the contraction had reached its height, which did not always coincide with the cessation of the stimulation, dilatation began but always there remained behind a certain amount of constriction. This residual contraction increased with succeeding stimuli and became constant as did L.P. Once constant, it required a considerable time, usually 30 to 60 mins., to disappear completely, and with the return of the vein to its normal flabby condition, the L.P. etc. reverted to their old values. On again stimulating, facilitation appeared. It would seem therefore that the type of contraction obtained depends upon the amount of the tone of the vein wall. The difference of tone is difficult to explain. It does not concern the species as it occurred in dogs, cats and rabbits, neither is it governed by age or sex. It does not depend on shock as it is met with in animals reacting quite differently to the operation. The type of anæsthetic has nothing to do with it, and it occurs after decerebration. It was not due to accidental interference with adrenaline secretion in removing the intestines, since stimulation of the nerves without touching the abdomen gave the same results. Furthermore the injection of adrenaline into type (B) animals and stimulation during the rise in B.P. caused no change. In any animal, however, all the veins belong to the same type which points to the difference being resident in the individual animals. The type difference is interesting as it may possibly be a factor in the liability to varicose veins which is so marked in certain human beings.

As to the "facilitation" process, all the evidence points to its being situated in the vein. The nervous factor was easily excluded by stimulating different segments of the L.S.C. or ant. crural nerve, and by the use of non-polarisable electrodes. The account I have given shows that it is fundamentally related to the tone of the vein wall.

Attention was now directed to the veins of other parts. Those of the fore-limb were found to give contractions similar in all respects to the veins of the hind limb. The type difference was also encountered and generally the upper limb veins show weaker contraction and longer L.P. than those of the hind limb. As for example, in one type (B) animal three stimulations of 60 secs. were necessary to obliterate the int. saphenous vein, while after five of the same strength and duration, the vein of the fore-arm did not completely collapse. (In each case the sympathetic cord was stimulated.) In the ext. jugular vein, a slight amount of contraction is sometimes seen at the upper end on stimulation of the upper dorsal and cervical sympathetic, but the small superficial veins usually contract on such stimulation. Using method 2 in dogs, the inf. mesenteric vein was found to contract, on stimulation of the nerve accompanying the inf. mesenteric artery, thus confirming Hooker's results. In cats and rabbits, the vein is small and cannot readily be perfused but after tying the tributaries the vein visibly constricted on stimulation. Here however no intensity of stimulation gives the complete obliteration, which one gets in the lower limb. In the sup. mesenteric vein by method 2 contraction was obtained in three cats on stimulating the right splanchnic nerve; with the left splanchnic there was no contraction. Contraction can be seen in the smaller veins of the mesenteric system and it would seem that the smaller the vein, the more complete the contraction. In the vena cava and its large tributaries returning the blood from the limbs and trunk, I have never succeeded in obtaining contractions. The sympathetic cord, lumbar and dorsal, the hind end of the spinal cord cut in the cervical region, were stimulated with all strengths of faradic currents and the results tested by methods 1 and 3, but in no case was contraction obtained.

When a superficial vein receives a deep tributary returning blood from the muscles, contraction always stops short somewhat distal to the junction point. The marked contrast between the parts on each side of the junction is evident in the int. saphenous and dorso-radial veins and can be most strikingly shown by perfusing the int. saphenous vein with adrenaline 1 to 1,000,000 with the exit cannula in the iliac vein. The deep veins themselves and the short branches connecting the latter with the superficial veins always remain uncontracted.

The effects of different forms of stimulus. Different forms of direct and indirect stimulation were tried on type (A) veins and on those of type (B) when they had reached a constant condition. A single make or break of a constant current up to 20 volts applied directly or through

the nerve never gave contraction, and the same strengths of current passing for durations from 1 sec. to 2 mins. gave negative results. Single induction shocks of increasing strength were tried without avail. Condenser discharges were also used by an arrangement similar to that of Lucas(9) with the introduction of a device for making and breaking the charging circuit. The voltage (V) varied from fractions of a volt to 20 volts, the capacity (C) from ·01 to 1 micro-farad. The experiments were rough, but were sufficient to show that with the same amount of energy per stimulus, those with low voltage and high capacity—in other words, stimuli with slow rate of rise and fall—were more efficient: *e.g.* using a $C \times V = 0{\cdot}2$, a series of 20 stimuli at a rate of 60 per sec. with $V = 0{\cdot}2$ and $C = 1{\cdot}0$ caused a contraction, but with the same number of stimuli at the same rate, when $V = 0{\cdot}4$ and $C = 0{\cdot}5$ no contraction appeared.

Mechanical stimulation of the nerve by pinching with the forceps or tearing it through is the most efficient of all forms of indirect stimulation. In one type (B) vein 20 secs. L.P., with weak coil current pinching the nerve three times caused after a L.P. of 30 secs. a strong contraction lasting 10 mins. The most lasting contraction is seen on direct stimulation of the vein by passing a ligature round it or by tearing its sheath away. In each case a local contraction is set up, which may continue up to 60 mins. Direct stimulation by strong coil currents always produces a local contraction, even when the vein ceases to respond to stimulation of its sympathetic supply after injection of ergotoxine. No difference is noticed in the L.P. on direct stimulation of a type (B) vein before and after injection of ergotoxine.

The origin and course of the veno-motor fibres.

This point was chiefly studied in the lower limb, but attempts were made to determine approximately the course in the upper limb and the intestines. In the lower limb, it was found while stimulating the lumbar sympathetic cord, that a contraction took place with segments 4–5, 5–6 and 6–7, with segments 2–3 contraction was never found, but with the segment between the 7th lumbar ganglion and the first sacral contraction usually took place in the ext. saphenous vein. To trace the origin of these from the spinal cord, ten experiments were carried out (7 cats, 2 dogs, and 1 rabbit). The dura was opened and silk threads were passed round the ant. and post. roots and carefully tied. The roots were then cut between the ligature and cord, and the segments of the cord removed piecemeal, to avoid current spread and reflex action.

The sympathetic cord was dissected out to serve as a test of the activity of the veins. The results are given in Table III.

Table III. Preganglionic fibres.

	1st L.	2nd L.	3rd L.	4th L.	5th L.
Cat 1.	None	Thigh, leg	Leg	Doubtful	None
„ 2.	„	Thigh	Dorsum pedis	Dorsum pedis	„
„ 3.	„	Upper int. S.V.	All int. S.V.	D.P. and ext. saph. veins	„
„ 4.	„	„	„	Ext. saph.	„
„ 5.	„	„	„	„	„
„ 6.	„	„	„	„	„
„ 7.	„	„	„	„	„
Dog 1.	„	Thigh	„	D.P., E.S.V.	„
„ 2.	„	„	„	„	„
Rabbit	„	„	All vein	E.S.V.	„

It is clear from Table III that the preganglionic fibres leave the cord chiefly in the anterior roots of the 2nd and 3rd lumbar. None was ever found in the first lumbar root; when fibres were present in the 4th lumbar they usually went to the ext. saphenous vein and the dorsum pedis. The three types, cat, dog and rabbit agree as to the course of these fibres.

The course of the postganglionic fibres was determined according to the two methods devised by Langley(10) in his work on the sympathetic system: the nicotine method, and that in which the sympathetic chain is cut above and below the ganglion, and thus the postganglion fibres stimulated. With the nicotine method, the cell stations could be immediately seen as a careful painting of the ganglion with the nicotine solution always produced a transient contraction of the vein. The results are summarised in Table IV.

Table IV. Postganglionic fibres to spinal nerves.

	L. 6	L. 7	S. 1	S. 2
Cat 1	Trace	Maximum	Marked	None
„ 2	Maximum	Marked	Slight	„
„ 3	None	Maximum	Marked	Doubtful
„ 4	Trace	Marked	Maximum	None
„ 5	None	Maximum	Marked	Doubtful
„ 6	„	„	„	None
„ 7	„	„	„	„
Dog 1	Trace	„	As L. 7	Doubtful
„ 2	„	„	„	„
Rabbit	Slight	„	Marked	„

Table IV shows that in the lower limb, the postganglionic fibres leave the sympathetic chiefly in the grey rami to the 7th lumbar and 1st sacral nerves. Some are always present in the 6th lumbar and in one case (cat 2) this nerve showed a maximum effect. Here subsequent dissection showed that the lumbar plexus was an example of Langley's anterior type. The grey ramus to the 2nd sacral being so short, I do not

lay any stress on the results of its stimulation, but in cases marked doubtful some contraction of the ext. saphenous vein was noticed. In general Tables III and IV bear out the results of Bancroft, the minor points of difference being due to the fact that he chiefly studied the ext. saphenous vein. It may be remarked that the postganglionic fibres for the int. saphenous vein run in the ant. crural nerve, while those of the ext. saphenous run in the sciatic. In no case did I find evidence of the existence of a plexus spreading along the veins from the nervous centres in the abdomen.

Four experiments on cats were made on the fore-limb. The dorsal sympathetic cord was exposed and dissected to the 10th ganglion. On stimulating it at any part between the stellate ganglion and the 8th contraction of the dorsal-radial vein and its continuation upwards always occurred. The spinal roots were then stimulated, and in each of the four cats the 7th root gave the strongest contraction. Nos. 6 and 8 also caused a contraction, but I could not obtain any with roots, above 6 or below 8. The cell stations are almost exclusively in the stellate and the postganglionic fibres to the dorso-radial run in the musculo-spiral nerve. The veins on the ulnar side of the fore-leg are very difficult to observe as they practically disappear on opening the skin. The chief nerve to these veins is the 8th dorsal. The superficial veins of the face and the ear have their cell stations chiefly in the superior cervical ganglion but here also the results are not satisfactory.

In the case of the sup. and inf. mesenteric veins, the nerve supply in each case is derived from the corresponding ganglion. A full account of these plexuses will be found in the work of Langley and Anderson(20).

Dilator fibres. When I first met veins of the (B) type, I thought that their peculiarity might be explained by the presence of vaso-dilator fibres more rapidly fatigued than the constrictor fibres. If this were true, one would expect the first result of nerve stimulation to be dilatation but no such result was ever obtained by the most careful use of method 3. In addition, in the experiments on the veins of the hind limb, I have always stimulated the post-lumbar and 1st sacral roots, with the vein in a normal condition, and in a contracted state, as a result of a previous stimulation of its motor supply. In only one case—a dog—have I found the post. roots to produce dilatation; in this, stimulation of the first sacral post. root gave undoubted dilatation on five repetitions, especially marked where the vein was contracted. The stimulation used was the one Stricker and later Bayliss(12) found so efficient in

their work on vaso-dilator fibres, viz.: mechanical stimulation by pinching the nerve.

A curious phenomenon which I met with in at least four cats may be mentioned here. In type (B) veins where they were slowly beginning to dilate after a contraction, it was found that pulling up the sympathetic chain to place it on the electrodes, caused immediate and complete relaxation of the vein. I have tried to reproduce this same phenomenon with other types of stimulation (electric) with negative results.

The effect of drugs.

The researches dealing with this point are confined to those of Gunn and Chevasse[13] and of Edmunds[14]. The former find that circular strips of sheep's veins—ext. jugular and mesenteric—give strong contractions with adrenaline, 1 in 100,000, while with the vena-cava the result is much less marked. Edmunds tried strips of the portal vein of dogs and got contractions, but does not state the strength of the adrenaline used.

My experiments were chiefly with adrenaline, but I have also tried ergotoxine and histamine. Three methods were used, (*a*) local application of solution of the drug to the vein by a thin capiliary tube, (*b*) injection of the drug into the circulation, (*c*) perfusing the vein with solutions of the drug. For perfusion a Mines cannula was used, one inflow tube being attached to the Ringer reservoir, the other to the reservoir containing Ringer plus the drug to be tested. For the present purpose, the results by (*a*) though very rough, are most fruitful as they show the marked difference between the veins of the different areas which was noticed on electrical stimulation. From the results of the several experiments, Table V is drawn.

The veins vary somewhat from one animal to another, but Table V gives an approximate idea of the sensitivity to adrenaline. With ergotoxine and histamine, much the same kind of results were obtained. With 1 in 1,000,000 or even 5,000,000, the superficial veins contract completely, while with 1 in 100,000, the cava and tributary veins show no effect.

The contraction passed off at varying periods after the application of all three drugs. After adrenaline, a further application of this drug produced a contraction but after ergotoxine neither adrenaline nor stimulation of the nerve gave constriction, and although I carefully inspected the veins under these conditions, I could find no evidence of dilatation. The ergotoxine contraction is very slow in disappearing,

TABLE V. Effects of adrenaline on different veins.

Vein	Strength of drug	Result
Int. saph. below knee	1 in 1,000,000	Complete obliteration of lumen
Int. saph. in thigh	" "	" " "
Int. saph. above entry of muscular branches	" "	None
" " "	" 100,000	Constriction
" " "	" 1,000	Diminution to half
Deep muscular branch	" 100,000	None
" " "	" 1,000	Very little change
Ext. saphenous	" 1,000,000	Complete obliteration
Ext. saph. near entry through muscles	" "	None
" "	" 100,000	Slight
Common iliac	" "	Very slight
" "	" 1,000	Slight
Inf. vena cava, point above origin	" 100,000	None visible
" " " "	" 1,000	Doubtful
Inf. cava above kidney	" "	None visible
Dorsal radial	" 1,000,000	Complete obliteration
Branch from D.R. to deep veins at anticubital fossa	" 100,000	None visible
Vena anti-cephalica	" 1,000,000	Marked contraction
Vena anti-ceph. near deep entry	" 100,000	Slight
Jugular, at submax. gland	" 1,000,000	Very slight
" " "	" 100,000	Contraction
Jugular middle	" "	None or very slight
Inf. mesenteric	" "	Strong contraction
Sup. mesenteric	" "	Contraction
Portal	" "	Fair contraction

and the paralysis of the sympathetic endings remains for a period of 30 to 60 mins.

By injecting the drugs into the circulation through the jugular vein one gets the same type of result. With adrenaline, I have not seen doses up to ·01 mg. produce any effect. With doses of ·02–·04 mg. however, the superficial veins are almost completely closed. The constriction comes on 60 to 120 secs. after the beginning of the rise in B.P. and continues for four to five minutes after the B.P. has returned to normal. Here again the difference in reaction of the different veins is marked. In the int. saphenous, the contrast between the contracted portion up to the entrance of the deep muscular veins, and the uncontracted portion above this point, is very striking. No change is visible in the cava or any of its large limb tributaries, but the intestinal veins, especially the smaller ones, are markedly smaller. These results can be repeated several times. With ergotoxine also, a larger dose is required than is necessary to affect the B.P., but with a dose of ·5 mg. per kilo a contraction of the veins occurs which has the same general relation to the B.P. as with adrenaline, and also picks out the veins in the same way. The dilatation after this drug is even slower than after adrenaline,

and neither a large dose of adrenaline nor stimulation of the nerve supply can now produce a contraction. Histamine in doses ·01 mg. per kilo which produce a marked fall in B.P. seems to have little or no effect on the veins.

In perfusing the veins a complication soon arises. When the Ringer has been passing through for periods ranging from 15 to 60 mins., the power of contractility of the vein ceases, a point which must not be forgotten when judging the results. Perfusion with strengths of 1 in 1,000,000 of adrenaline, ergotoxine and histamine, always completely closed the int. saphenous and dorso-radial veins. The drugs in a strength of 1 in 100,000 had no effect on the vena cava, but in this strength reduced the outflow from the inf. and sup. mesenteric to about one-fourth. The effect of perfusing adrenaline after ergotoxine was tried, but after either of these drugs, the contraction is so marked that the normal Ringer has to be forced through the vessel in order to establish the flow, even then the contraction is slow in disappearing (5–10 mins.). During this period, neither adrenaline nor nerve stimulation causes contraction, but after complete recovery, the vessel responds to both. I could not obtain decisive evidence of a dilatation being produced by either of those two agents during the recovery period.

Central control and reflex action.

The question of a tonus existing in the venous side of the circulation and its dependence on a nervous connection with the central nervous system has, like all other aspects of the physiology of these vessels, received little attention. About the only discussion on the subject is that of Tigerstedt in his *Physiologie der Kreislauf*, where he points out, from the results obtained by Goltz, that this factor must be of some importance. Hooker's recent experiments, already mentioned, point also in this direction. By the method of perfusing the vein, and recording its outflow, one has an easy means of obtaining unequivocal evidence.

The immediate result of the section of the nerve is a decrease in flow lasting for 30–90 secs. followed by a marked increase. In one case the normal flow was 5·2 c.c. per min., for the minute immediately after the nerve section it was 4·0 c.c., for the next minute 9·8 c.c. and for the 8th minute 9·5 c.c. Thus the mechanical stimulation caused by the section of the nerve gives rise to contraction. This occurs both in the cat and the dog, and as I have said I have not found any vaso-dilator action in the cat. It may then be concluded that the subsequent dilatation is due to cutting off the vaso-constrictor impulses and not to active

vaso-dilation. The dilatation due to nerve section is easily visible by comparing the veins of opposite legs, in one of which the ant. crural nerve has been cut. The vein of the upper limb behaves similarly on cutting the sympathetic cord below the stellate ganglion, and as Hooker has already shown the same results are obtained with the inf. mesenteric on cutting the nerve with the inf. mesenteric artery, here the primary constrictor effect is more prolonged. Despite this undoubted evidence of central tone, the superficial veins, as judged by the response of the int. saphenous and veins of the upper limb, do not seem to be involved, at least to any noteworthy extent, in reflex changes. No effect was found on stimulating on either side the central end of the vagus, the sciatic or the sensory nerves of the fore-limb, although each caused the cus-

Fig. 4. Inf. mesenteric vein, reflex effects of vagus stimulation.

tomary rise of blood-pressure. In all cases subsequent stimulation of the peripheral nerves caused contraction. But with the inf. mesenteric vein (dog) the results are in striking contrast. Here changes in the general vascular tone produced reflexly are always accompanied by corresponding changes in the veins. Any influence causing a rise in general blood-pressure, for instance, stimulation of the central end of the sciatic or vagus or asphyxia, causes a corresponding decrease in the calibre of the vein (Fig. 4) shows the effect of stimulation of the central end of the vagus. The B.P. tracing is not included, but the constriction of the arterioles slightly preceded that of the veins. In dogs stimulation of the central end of the vagus nerve gave usually a rise in blood-pressure, a fall was only obtained in two out of some twelve

attempts. In each of these the inf. mesenteric vein was dilated; in one case the flow increased from 10 to 17 c.c. per min.

The exact path of the reflex on the mesenteric veins I have not determined. It does not take place if the spinal cord is cut below the bulb. Cutting the nerve fibres accompanying the inf. mesenteric artery abolishes it. The same result is obtained by painting the inf. mesenteric ganglion with nicotine. But the reflex is unimpaired if the L.S.C. is cut between the 1st and 2nd, and 6th and 7th, lumbar ganglia, or if the lumbar sympathetic ganglia are all painted with nicotine. Evidently then the effector arc of the reflex is situated in nerve fibres leaving the cord in the upper lumbar nerve roots, probably 2, 3 and 4, with cell stations situated not in the sympathetic chain, but in the inf. mesenteric ganglion. The upper portion of the vein, where it begins to leave the intestine to join up the superior mesenteric vein, is also supplied with fibres from the sup. mesenteric ganglion.

This contrast between the response of the superficial and mesenteric veins to reflex stimuli, is strongly borne out by their behaviour under normal conditions. If the abdomen is opened and the veins there and in the leg inspected while a rise in B.P. is produced by stimulating the central end of the sciatic, it will be seen that the superficial leg veins are not affected, while the intestinal veins especially the smaller tributaries are practically closed. Central vaso-motor stimulation by asphyxia gives the same results.

Since the superficial veins have a central tone, it would be remarkable if they could not be reflexly influenced. It is probable that they can be by activating the appropriate receptor. On several occasions it has been noticed in dogs and cats, that immediately after the skin has been removed from the superficial veins of the leg, those veins contract till they completely obliterate their lumen, from the dorsum pedis right up to the entrance of the deep leg vessels. If the vessels are covered with the skin or a warm pad, the contraction disappears in a few minutes. If the skin and pad are again removed another contraction appears, and again disappears on warming. This phenomenon however is not present in all animals. In some, especially of type (A), it can be repeated almost indefinitely. In nearly all it is met with in the fore-leg. If before the skin or pad is removed, the ant. crural nerve be cut, no constriction occurs, and if the contraction be produced, cutting the nerve abolishes it; section of the sciatic has no effect on either result. If the vein be kept continuously uncovered, it returns to a normal size and then covering it with skin or a warm pad with subsequent uncovering causes

no change. Furthermore the effect is local for while the vein of the right leg is laid bare and consequently contracted, that of the left leg on inspection is seen to be of normal size. It seems clear that this contraction is reflex in character, the receptor unit being probably a temperature ending in the superficial tissues near the vessel of the leg concerned.

It was observed in some cats where shivering was marked that during the fits, the leg veins contracted and again dilated when the animal was warmed. This occurred when the vein was quite insensitive to covering and uncovering, which points to the receptor as being the delicate part of the reflex. Some trials were made to perfuse the corpus striatum of the rabbit with hot and cold water, as in Barbour's experiments(15). The experiments were done under anæsthesia and were accordingly complicated by the accompanying fall in body temperature. Still the dilating and constricting effects of hot and cold perfusion in the veins of the rabbit's ears were very marked and appeared 10 to 15 mins. before the temperature changes. The response to variations of the temperature of the perfusion fluid was also tried; a drop from 37° C. to 27° C. usually produced a 50 p.c. decrease in the outflow. As cutting the nerve did not cause an appreciable difference, the temperature effect must be a purely local one.

Some observation on perfusion fluids. It has already been mentioned that after the perfusion fluid has been allowed to pass through the vein for some time the vein ceases to respond to the action of drugs or to nervous stimulation. The length of time necessary to reach this stage varies from vein to vein, but rarely exceeds 60 mins. and frequently is much less. The onset of the condition is heralded by a slight but noticeable constriction of the vein, which gives way in varying time to dilatation relative to the normal condition. When the dilatation has become established, no strength of stimulus or adrenaline even ·1 p.c. in the int. saphenous can provoke a constriction.

The loss of irritability is not due to fatigue since the vein in the body with blood flowing through it remains responsive for hours to nerve stimulation. The loss of irritability takes place at about the same rate in the veins of the two hind limbs, but rather more quickly in the veins of the fore-limb than in those of the hind limb. It is quicker in veins of type (B) than in those of type (A); type (B) veins may cease to respond to faradic stimulation and to ·002 p.c. adrenaline in 10 mins. The more rapid the perfusion the quicker the loss of irritability. The experiments show that something necessary for contraction is rapidly washed out of the unstriated muscle of the veins.

I have made some experiments with modifications of the perfusing fluid to see if the irritability could be retained for longer periods than with Ringer's fluid. This contained NaCl ·9 p.c., $CaCl_2$ ·024 p.c., KCl ·042 p.c., and $NaHCO_3$ ·015 p.c., the water was metal distilled, the solution had a p.H of about 8. Using glass distilled water, and bubbling O_2 through the fluid, and making the p.H about 7, had no appreciable effect. Addition of 6 p.c. gum or of ·1 p.c. glucose seemed to be slightly beneficial. The addition of lecithin to make ·01 p.c. made no difference to the length of survival though it restored the soft natural appearance; addition of an ether extract of red blood cells had the same result. The only suggestion, that some modification of the perfusing fluid might retain the irritability, was that a Ringer's fluid, in making up which a rather impure NaCl was used, kept the veins in a contractile condition for three hours. Perfusion with defibrinated ox blood rapidly destroyed the irritability.

Summary.

1. The veins of the animals investigated (dog, cat, rabbit) can be roughly divided into three groups:

(*a*) The superficial veins returning blood from the skin. These receive sympathetic nerve fibres. The veins of the hind limb receive them from the 2nd to the 4th L. nerves inclusive chiefly by way of the 7th L. and 1st S. ganglia, those of the fore-limb from the 6th to the 8th D. with cell stations mainly in the stellate ganglion. They react differently in different individuals, in some they contract on the first weak nerve stimulation, in others they only contract after repeated stimulation. These veins were not found to react in the usual vascular reflexes, but probably they take part in temperature reflexes.

(*b*) The mesenteric veins. These are supplied with sympathetic nerves having their cell stations in the ganglia of the solar plexus, and the inf. mesenteric ganglion. They are influenced reflexly in the same way as the arterioles.

(*c*) The veins from the muscles and the vena cava. No effect was found in these.

2. Adrenaline 1 in 1,000,000 causes marked contraction in the veins of groups (*a*) and (*b*), but is practically inert in those of group (*c*).

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THE SURVIVAL OF MOTILITY IN MAMMALIAN SPERMATOZOA.

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One of the problems of great practical importance in animal breeding is that of being able to keep spermatozoa viable for a sufficient length of time, so that they may be transported and used for distant insemination. Up to now, artificial insemination has been practised only with semen immediately after ejaculation or at most an hour or two after passage. My attention was directed to this problem by Dr F. H. A. Marshall(1), who had already done some experiments in this direction, and I undertook to ascertain what were the conditions under which survival could best be prolonged. We know that mammalian spermatozoa remain viable outside the male organism for periods up to twenty days(2, 3), so that it ought to be quite possible by providing a suitable environment to keep them potent for some considerable length of time.

Iwanoff(4) found that the movements of spermatozoa continued for some days when the excised testicle was immersed in Locke's solution. When the semen itself was used the duration of motility was shorter. Survival was promoted by a low temperature. Poyarkov(5) found that one part of Locke's solution diluted with nine parts of isotonic glucose solution was more efficient in prolonging motility than the salt solution alone. Ochi(6) and Sato(7) confirm this result and state that oxygenation of the solution is beneficial. None of these investigators have paid any attention to the effect of buffer salts or the real reaction on survival.

Method. The method in my experiments was as follows: A rabbit was killed and the epididymis removed at once and placed in a short wide tube of 25 c.c. capacity, containing 15 c.c. of the solution to be investigated. The tube was enclosed in a larger tube which was packed in ice in a large wide-mouthed thermos flask. The solution preserved a temperature of 1–2° C. for days with the use of very little ice. The motility of the sperms was observed by taking a small amount from the

cut end of the epididymis with a pipette and placing it on a ring microscopic slide which was enclosed in a warm stage at 37° C. A low power was sufficient to determine the motility.

The first experiments were made to ascertain the effect of adding buffer salts to a solution of the Locke type. In the first experiments, Burroughs and Wellcome's soloids were employed which gave a solution containing approximately ·72 p.c. NaCl, ·016 of KCl and $CaCl_2$, ·008 p.c. of $NaHCO_3$ and ·08 p.c. of glucose. To 100 c.c. of this solution were added 2 c.c. of M/8 KH_2PO_4 and 23 c.c. of M/15 Na_2HPO_4. The amounts were used as the results of titrations which gave a mixture having a hydrogen ion exponent of about 7·3. The reactions of the solution were determined colorimetrically by comparison with carefully checked phosphate standards. In the second series of experiments the effect of diluting the salt solution with an equal volume of a M/8 solution of glucose was investigated. The effect of oxygenating the mixture was also tried. The results are given in Table I.

TABLE I.

Soloid Solution	P.H.	1 day	2 days	3 days	4 days	5 days	6 days
1. Control	6·1	Very active	—	0	—	Dead	—
2. Buffered... ...	7·4	Very active	—	Majority active	—	Few active	—
3. Control + O_2 ...	6·6	—	Few moderately active	—	Dead	—	—
4. Buffered + O_2 ...	6·4	—	Many active	—	Few active	—	—
5. Control and glucose	6·4	—	6	—	0	—	0
6. Control and glucose buffered...	7·4	—	Active	—	Majority active	—	Few active

The hydrogen ion concentration was determined in the oxygen experiments before passing in the gas. This was also the case in all experiments except one in Table II. It was found that the passage of oxygen increased the alkalinity of the solutions by decomposing the bicarbonate. In the last experiment the bicarbonate was added after treatment with the gas. It was found that the decomposition of the bicarbonate and consequent rise in alkalinity had a pronounced effect on survival, for in none of the preparations was the motility of the isolated sperms so prolonged as in the last experiment. The results of the second series of experiments are set down in tabular form in Table II.

Experiments made to fecundate rabbits with spermatozoa which had been kept in salt and glucose solutions were unsuccessful. There

TABLE II.

Solution buffered and glucose	P.H.	1 day	2 days	3 days	4 days	5 days	6 days	9 days
1. Locke's sol. (epididymis)	7·4	Very active	Little motility	Active	Active	—	?	—
2. Locke's sol. + O_2 (epididymis)	7·4	Very active	Quite active	Active	Greater than control	—	Active	—
3. Tyrode's sol. (suspension)	7·5	Not very active	No action	Some action	—	Dead		—
4. Tyrode's sol. + O_2 (suspension)	7·5	Very highly active	Very active	Many active	—	Few moving	—	—
5. Tyrode's sol. (epididymis)	7·5	—	—	—	—	Not very active	—	—
6. Tyrode's sol. (epididymis)	7·5	—	—	—	—	Not very active	—	—
7. Tyrode's sol.	7·5	—	—	—	Few motile	—	—	—
8. Tyrode's sol. + O_2 ...	7·5	—	—	—	Vigorous movement	—	—	—
9. Tyrode sol. + O_2 ...	—	—	Active	Active	—	—	—	—
10. Tyrode sol. Serum (1–10) + O_2	—	—	—	Not so active	—	—	—	—
11. Tyrode sol. and serum (1–6) + O_2	—	—	Not so active	—	—	—	—	—
12. Tyrode sol. + O_2 buffered with NaH_2PO_4	7·5	—	—	Quite active	No activity	—	—	—
13. Tyrode sol. + O_2 (glass distilled water)	—	—	—	—	—	—	—	—
14. Tyrode sol. + O_2, bicarbonate added after adjustment	7·4	—	—	Most highly active	Active	—	—	Still motile

were certain signs of pregnancy, such as fleecing and swelling of the abdomen, but no young were obtained. Iwanoff points out that rabbits are very unsatisfactory animals for artificial insemination experiments, owing to the frequency with which they devour their young under laboratory conditions of breeding.

SUMMARY.

The motility of rabbit spermatozoa can be preserved for at least nine days by placing the juice of the epididymis in a Tyrode solution which has been buffered and to which glucose has been added. The solution is adjusted to a *p*.H of about 7·4. Oxygen is passed in and a suitable amount of sodium bicarbonate added. The preparation must be kept at a temperature near the freezing point of water.

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STUDIES ON RESPIRATION AND CIRCULATION IN THE CAT. II. The effects of hæmorrhage and transfusion of gum-saline solution.

By Y. DOI.

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GESELL(1) found that the rate of blood flow from the submaxillary gland in the dog was not directly proportional to the arterial pressure, a relatively small fall of pressure from the normal height caused a greater decrease in this blood flow than that caused by an equal fall starting from a lower level, and Bayliss made a similar observation on the rate of flow of blood from the deep femoral vein. At the suggestion of Mr Barcroft I have studied the effect of variation of blood-pressure at different levels on the gaseous exchange and minute volume in the cat, the blood-pressure being changed by hæmorrhage and intravenous injection.

Methods. The methods used and the factors measured are the same as those given in my previous paper(3); it will be superfluous, therefore, to describe the details here. The left carotid arterial pressure was recorded. Blood was taken from the right femoral artery, and the solution was injected into the right femoral vein. The blood samples for gas analysis were taken from the left femoral artery and directly from the heart. When the heart was punctured at a very low blood-pressure, such as 40 mm. Hg, the animal died in most cases, so that all experiments were made at a higher blood-pressure than 50 mm. Hg. The anæsthetic used was urethane. In some experiments, injections of saline solution were made subsequent to the bleedings and the observations repeated. In such cases the concentration of hæmoglobin which carries the oxygen is reduced by the injection so that though the pressure is raised to the normal height the oxygen supply is not necessarily the same as it originally was.

The solutions injected at about 38° C. were Ringer's solution and 6 p.c. gum-saline solution—a comparison being made between the effects of these solutions. Since Ringer's solution has a low viscosity, which is practically the same as that of water, an equal volume of this solution does not raise the blood-pressure to its normal level, so that an amount of Ringer's solution, greater than that of blood taken out, was injected.

The amount of gum-saline solution, which was injected after hæmorrhage, was the same as that of blood taken out. Every possible care was taken to maintain the temperature of the cat at the normal level throughout the experiment.

Results. At low blood-pressures the amount of oxygen taken up per minute by the animal and the minute volume are both less than those at higher ones. As is seen in Table I, the former was 20·2 c.c. and the latter 317 c.c. at 110 mm. Hg blood-pressure. When the blood-pressure was lowered to 89 mm. Hg, 76 mm. Hg, and 54 mm. Hg, the amounts of oxygen taken up per minute by the animal were 17·74 c.c., 16·26 c.c., and 12·80 c.c. respectively, and the minute volumes were 221 c.c., 195 c.c. and 147 c.c. respectively. These figures show that the percentage decrease of the amount of oxygen taken up and of the minute volume at a low blood-pressure are not the same.

TABLE I. Urethanized cat, 2·75 kilo.

	Normal state	20 mins. after bleeding 10 c.c.	20 mins. after bleeding 15 c.c.	20 mins. after bleeding 20 c.c.
Blood-pressure, mm. Hg	110·0	89·0	76·0	54·0
Volume of resp. per min., c.c.	600·0	710·0	740·0	780·0
Volume of single resp., c.c.	19·4	17·3	16·4	15·6
Output of each heart beat, c.c.	1·98	1·49	1·43	1·13
Oxygen taken up in c.c. per min. (0° C., 760 mm. Hg and dry)	20·2	17·74	16·26	12·8
P.c. saturation of O-Hb in arterial blood ...	89·0	88·4	88·2	89·2
P.c. saturation of O-Hb in venous blood ...	43·6	33·9	31·3	29·0
Difference between O_2 in 100 c.c. arterial and venous blood	6·39	8·0	8·34	8·92
Minute volume, c.c.	317·0	221·0	195·0	147·0

The decrease of the amount of oxygen taken in is less than when the blood-pressure falls about 19 p.c., 30 p.c. and 50 p.c. from the normal heights, the decrease of the former is about 12 p.c., 20 p.c. and 37 p.c., and the decrease of the latter is 30 p.c., 38 p.c. and 54 p.c. respectively. It is seen from these figures, too, that the first fall of the blood-pressure from the normal height has more effect than a further fall from a lower level. The greatest effect observed in my experiments was on an occasion when the blood-pressure fell about 30 p.c. from the normal height, the decrease of the minute volume was nearly 53 p.c. These results, however, are not so striking as those found by Gesell and Bayliss.

It is well known that when an animal loses blood, the respiration increases in frequency and it becomes shallow. These results followed in my experiments. The output of each heart beat decreases with a fall of the blood-pressure. Table II, which is one of four concordant experi-

ments, shows the effect of an injection of Ringer's solution and gum-saline solution. When Ringer's solution was injected into the animal

TABLE II. Urethanized cat, 2·25 kilo.

	Normal state	20 mins. after bleeding 35 c.c.	10 mins. after injection of Ringer's sol. 60 c.c.	1 hour after injection	10 mins. after gum-saline injection 35 c.c.
Blood-pressure, mm. Hg.	100·0	56·6	94·0	59·0	104·0
Volume of resp. per min., c.c.	330·0	370·0	540·0	570·0	530·0
Volume of single resp., c.c.	8·93	8·05	9·65	8·15	8·884
Output of each heart beat, c.c. ...	1·31	0·8	1·45	·7	1·15
Oxygen taken up in c.c. per min. (0° C. 760 mm. Hg and dry)	13·25	9·1	17·8	8·86	12·2
P.c. saturation of O-Hb in arterial blood	88·6	89·7	79·3	89·2	86·2
P.c. saturation of O-Hb in venous blood	46·8	32·2	31·5	29·8	44·2
Difference between O_2 in 100 c.c. arterial and venous blood	5·97	8·23	6·82	8·48	6·0
Minute volume, c.c.	222·0	110·0	261·0	105·0	203·0

after bleeding, the amount of oxygen taken in by the animal and the minute volume rose to a higher level than before hæmorrhage, but this did not last long and in an hour after the injection they decreased less than those before injection. The result is important as showing that the deficiency of oxidation during the period of low pressure can be made up subsequently. In the example given above, the blood-pressure, the amount of oxygen taken in, and the minute volume in the normal condition were 100 mm. Hg, 13·25 c.c. and 222 c.c. respectively; when 35 c.c. of blood were taken out, they were 56·6 mm. Hg, 9·1 c.c. and 110 c.c. respectively, and after injection of Ringer's solution they were increased to 94 mm. Hg, 17·8 c.c. and 261 c.c. respectively. But in an hour after the injection they decreased again to 59 mm. Hg, 8·86 c.c. and 105 c.c. respectively. At this time the blood-pressure was raised to 104 mm. Hg by injection of gum-saline solution, and the amount of oxygen taken in and the minute volume increased to 12·2 c.c. and 203 c.c. respectively, which were nearly the same as those in the normal state. In all experiments of this kind the blood-pressure began to fall very quickly after the last heart puncture and the cat died in 5–10 minutes. In one experiment, in which gum-saline solution was injected immediately after hæmorrhage, it was found that the blood-pressure, the amount of oxygen taken in, and the minute volume were increased to their normal values, and that after an hour all of these decreased about 15 p.c. of their normal value, but in four hours they regained their normal figures. (In my preliminary experiments no harm by an injection of gum-saline solution was seen.)

On those occasions in which puncture was followed by an immoderate drop of blood-pressure, I opened the chest and found that blood was coming out through the holes of the pericardium and the heart muscle, which were made by piercing with the needle of the syringe. It seems very likely, therefore, that an infusion of gum-saline solution raised the intraventricular pressure very high, so that fatal bleeding occurred through the holes made by the needle. In the other experiments the cats never died in consequence of the heart puncture.

By injecting Ringer's solution or gum-saline solution, the frequency of respiration and pulse as well as the volume of each respiration and of each heart beat were increased, even above the normal rate.

Summary.

When the blood-pressure of an animal is lowered to various levels by progressive bleeding, the amount of oxygen taken in the animal and its minute volume of blood flow through the lungs decrease with a fall of blood-pressure. The first fall of the blood-pressure from the normal height has greater effect on the minute volume per millimetre than the subsequent drop.

When the blood-pressure falls to a low level the decrease of the amount of oxygen taken in is less than that of the minute volume.

The frequency of respiration and pulse rate increase with a fall of blood-pressure, and the volume of each respiration and of each heart beat decrease with a fall of blood-pressure.

When the blood-pressure is lowered by hæmorrhage, injection of Ringer's solution or gum-saline solution raises it to normal height, and increases to normal values the amount of oxygen taken in and the minute volume. Gum-saline solution sustains these conditions for a longer time than does Ringer's fluid.

In conclusion I wish to express my warmest thanks to Mr Barcroft for his suggestion of this problem and for his kind and valuable advice throughout the course of these experiments. I am also indebted to Prof. Langley, in whose laboratory these experiments were made.

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THE FUNCTION OF THE URINARY TUBULES IN THE FROG. By MILDRED ATKINSON, G. A. CLARK and J. A. MENZIES.

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Indirect evidence justifies the belief that the first stage in the production of urine consists in the filtration of protein-free plasma through the glomerular capillaries and Bowman's capsule. But there are still differences of opinion as to the part played by the tubules. Bowman's theory does not account for the disappearance of chloride from the urine in chloride starvation while the percentage of chloride in the blood is maintained. Nor does it account for the fact that when the renal arteries of the frog are perfused with Ringer's fluid the urine formed is hypotonic to the perfusing fluid(1). Cushny's modification(2) of Ludwig's theory offers a rational explanation of the facts, but, from the nature of the case, it is based on indirect evidence. Cushny(2) assumes that the sole function of the tubules is the reabsorption from the glomerular filtrate of an "optimal fluid" of constant composition, containing sodium chloride, glucose and other "threshold substances" in the proportions in which they normally exist in the blood.

The experiments described in this paper were undertaken with a view to ascertaining whether in the frog any solid constituents pass into the urine through the tubular epithelium, or whether Cushny's view, that the sole function of the tubules is reabsorption, holds good.

In our experiments we have limited ourselves to the question whether urea, sodium sulphate or glucose passes through the tubule from blood to urine. We may note that it does not seem to us desirable to use the term "secretion" in this connection. The constituents of the urine, with the possible exception of hippuric acid, are pre-formed in the blood, and the cells of the tubules transmit them in one direction or the other.

The experiments were carried out by the perfusion method. Bainbridge, Collins and Menzies(1) have shown by injection methods that the efferent vessel from each glomerulus passes from the ventral to the dorsal surface of the kidney, where it opens into an irregular venous space. The venous spaces also receive branches of the renal portal vein,

and from them the intertubular capillaries run a fairly parallel course to the ventral surface of the kidney, where they unite to form the renal veins. There is a marked contrast between the narrow cylindrical efferent glomerular vessel and the wide irregular capillaries, explaining both the ease with which relatively large volumes of fluid flow through the intertubular vessels under low pressure, and the difficulty of forcing fluid from the renal portal vein backwards through the efferent vessel into the glomerulus under high pressure.

The following table shows the proportional flow of perfusing fluids through the renal arteries and renal portal veins, when simultaneous perfusion of Ringer's fluid is carried on through both, the arterial pressure being 24 to 26 cm. of water and the venous pressure 10 to 12 cm. of water. The amounts, in c.c., are for periods of five minutes.

Exp.	Arterial *plus* venous	Arterial only	Venous only
1	15	5	13
2	18·5	5·2	11
3	16·5	3·5	13
4	12·5	5·5	12·5
5	15	5	13
6	28	2·8	24·5
7	13	7·2	12·5
8	20	3	15
9	24·5	5·6	20·5
Average	18·2	4·7	15

The figures show that, under the conditions of the experiments, the amount of fluid by the venous perfusion was three times that by the arterial. A similar result was obtained indirectly. The arteries were perfused with Ringer's fluid, and the renal portal veins with Ringer containing ·33 p.c. of glucose. The perfusate contained ·26 p.c. of glucose, showing a proportion of arterial to venous perfusion of 7 to 26.

The method used was that described by Bainbridge and his coworkers(1), except that it was not thought necessary to remove the testes and fat-bodies. Oxygenated Ringer's fluid was perfused through the renal arteries, and Ringer's fluid containing urea, sodium sulphate or glucose, as the case might be, through the renal portal veins. The urea, sulphate or glucose could thus pass into the urine only through the walls of the tubules. In each series of experiments a second set was performed in which 2 p.c. of gum was added to the perfusing fluids, as suggested by Baylis. The addition of gum was of advantage chiefly in the experiments with urea. In these a considerable degree of œdema was produced in the kidneys when gum was not added, and was absent when gum was

used. The arterial perfusion pressure in the great majority of experiments was 24 cm. of water, and the venous pressure 11 to 12 cm. of water. These figures give no real indication of the pressures in the renal vessels, as this is modified by the small bore of the cannulæ. But it may be accepted that the pressure in the arteries was twice that in the renal portal veins. With these pressures the urine is entirely formed by filtration, and it ceases to be produced when the arterial perfusion is shut off.

Urea. Various tests were tried for the recognition of urea in quantities of Ringer's fluid comparable with the amount of urine usually available, that is, a fraction of a c.c. Effervescence with sodium hypobromite was found the most useful reaction. The test was easily carried out by introducing the reagent by means of a fine pipette into the cannula containing the urine. Attempts to estimate the amount of urea contained in the urine have not so far been successful.

Perfusion of the vessels of the kidney with Ringer's fluid alone resulted in the formation of urine which gave no effervescence with hypobromite.

Arterial perfusion with oxygenated Ringer's fluid containing 0·1 p.c. of urea gave urine which contained urea.

When the arteries were perfused with oxygenated Ringer's fluid, and the renal portal veins with Ringer's fluid containing urea, the urea invariably appeared in the urine. In the earlier experiments the amount of urea in the venous perfusing fluid was ·1 p.c.; in the later experiments, the great majority, the amount of urea was ·02 p.c. In one case the urine was evaporated to dryness, the residue extracted with acetone, the acetone extract filtered, again evaporated to dryness, and taken up with a drop of water. On mixing this solution on a slide with nitric acid minute crystals of urea nitrate were obtained.

Histologically, the perfused kidneys showed distension of Bowman's capsules and of the urinary tubules. The intertubular vessels were also wide, and the epithelial lining of the tubules was detached from the basement-membrane in many places. The addition of gum to the perfusing fluids prevented the detachment of the basement-membrane, but the Bowman's capsules and tubules still showed dilatation. The results as regards the presence of urea in the urine were unaffected by the presence of gum in the perfusing fluids. The following is a protocol of a typical experiment.

Male frog. Brain and cord pithed. R. aorta, mesenteric artery and thighs ligatured. Cannulæ in L. aorta, post-caval vein, anterior abdominal vein and both ureters.

Perfusion: arterial, oxygenated Ringer, pressure 24 cm. Renal portal, Ringer with ·02 p.c. urea, pressure 12 cm.

11 a.m. Dissection begun. 11.35 a.m. Dissection completed.

12.5 p.m. R. ureteral cannula ½ full (·25 c.c.). L. ½ full (·33 c.c.). Urine in each cannula effervesces with NaBrO.

Sodium sulphate. The test for the recognition of sulphate in the urine was carried out as follows. A small quantity of normal solution of barium chloride, slightly acidulated with hydrochloric acid, was placed in a small test-tube, and the urine dropped on to the surface with a pipette. It was found that a solution containing ·004 p.c. of sodium sulphate gave a decided precipitate.

On perfusion of the renal vessels with Ringer's fluid alone, no sulphate was found in the urine formed.

On perfusion of the renal arteries with oxygenated Ringer's fluid containing ·09 p.c. of sodium sulphate (·2 p.c. of the crystalline salt) the urine formed gave a precipitate with barium chloride.

On perfusion of the renal arteries with oxygenated Ringer's fluid, while the renal portal veins were perfused with Ringer's fluid containing ·09 p.c. of sodium sulphate, the urine formed was found to contain sulphate.

Cullis(4) found that sulphate passed into the urine from the intertubular vessels when the fluid perfusing these contained urea and sulphate. Our results confirm her observation.

Experiments in which gum was contained in the arterial perfusing fluid were not admissible, because gum solutions gave a slight precipitate with the barium salt. But experiments were carried out in which 2 p.c. gum was added to the fluid perfused through the renal portal veins, while the arteries were perfused with oxygenated Ringer, and these gave the same result as in the cases where gum was not used. The following is a protocol of one experiment.

Male frog. Ligatures and cannulæ as described in protocol of urea experiment. Arterial perfusion with oxygenated Ringer, pressure 26 cm. Renal portal perfusion with Ringer containing 2 p.c. gum and ·09 p.c. sodium sulphate, pressure 12 cm.

2.20 p.m. Dissection begun. 2.55 p.m. Dissection completed.

3.15 „ Both ureteral cannulæ full. Emptied. Sulphate in each.

3.35 „ „ „ „ „ „ „ „ L., R. doubtful.

3.55 „ „ „ „ „ „ „ „ each.

4.20 „ „ „ „ contain urine. „ „ „

The capacity of each cannula was 0·5 c.c.

The diuresis obtained in this experiment was unusually great, but the arterial pressure was 2 cm. higher than usual. The results as regards

volume of urine when sulphate was perfused have been very variable, and further work will be required before any explanation of the variability can be offered.

In one experiment an attempt was made to ascertain whether sulphate would diffuse into the kidney tissue. The aorta and renal portal vessels were perfused with Ringer's fluid for one hour, during which the body-cavity was filled with Ringer's fluid containing ·2 p.c. sodium sulphate. The urine formed during this time contained no sulphate.

The histological appearances of the kidneys after the sulphate experiments were similar to those after perfusion with urea, except that the basement-membrane was not as a rule detached from the tubular epithelium. The Bowman's capsules and the tubules were widely distended.

Glucose. Various methods of testing for traces of glucose in small quantities of fluid were tried, with the result that Benedict's solution was found to be most satisfactory. Cole's excellent glycerol and copper test was unsuitable for the volumes of urine available. Benedict's test gave an unequivocal positive result with small quantities of Ringer's fluid containing ·005 p.c. of glucose, and this may be considered as sufficiently delicate for our purpose.

Arterial perfusion with oxygenated Ringer's fluid containing ·1 to ·5 p.c. of glucose, with or without the addition of 2 p.c. gum, yields urine, as Hamburger(5) has shown, which contains glucose. In one experiment in which 2 c.c. of urine were obtained in 140 minutes from the two kidneys, an estimation showed that the urine contained approximately the same percentage of sugar as the perfusing fluid. This equality of concentration, together with the fact (as described below) that sugar does not enter the tubules from the intertubular vessels, shows that the sugar is filtered through the glomeruli.

Arterial perfusion with oxygenated Ringer's fluid, while the renal portal veins are perfused with Ringer's fluid containing ·1 to ·5 p.c. of glucose, yields urine free from glucose in detectable quantity. This result is unaffected by the presence or absence of gum in the perfusing fluids. On two occasions a perceptible haze was produced when the urine from one ureter was boiled with Benedict's solution. In both these cases the urine from the other ureter gave a negative result, and in both cases a second sample of urine from the same ureter was sugar-free. It was therefore believed that, during the insertion of the cannula into the ureter, a little perfusing fluid, which had leaked into the body-cavity, had entered the cannula. The number of experiments in which the urine

gave no reaction for sugar was sufficient to justify the belief that these two exceptional results were accidental. The following shows a typical experiment.

Male frog. Ligatures and cannulæ as described in urea experiment. Arterial perfusion: oxygenated Ringer, pressure 25 cm. Renal portal perfusion: Ringer with ·5 p.c. glucose, pressure 11 cm.

2.25 p.m. Dissection begun. 2.55 p.m. Dissection completed.

4 p.m. Both ureteral cannulæ full. No reduction of Benedict's solution by urine from either.

Not only is there no evidence in these experiments to show that glucose passes from the intertubular vessels into the urine, but other experiments (to be published later) point to the absorption of sugar from urine to intertubular vessels. The histological appearances after perfusion with glucose did not differ materially from those described in connection with perfusion with sulphate.

Conclusions.

The experiments described in this paper show that, in the frog, transmission of urea and of sulphate can take place from intertubular vessels to urine, whereas this is not the case with glucose. The facts already referred to in connection with chlorides indicate that chlorine normally passes in the cells from urine to intertubular vessels. Unless chlorides and sulphates are dealt with by different cells, which is improbable in the frog, there would thus appear to be cross-currents in each cell, Cl ions passing in one direction and SO_4 ions in the other.

Summary.

1. In perfusion experiments in the frog urea, sulphate and glucose are filtered through the glomerular membranes.

2. The cells of the tubules are capable of transmitting urea and sodium sulphate, but not glucose, from perfusing fluid to urine.

3. These results are not altered by the presence of 2 p.c. gum in the perfusing fluids, but the gum prevents the occurrence of œdema in the kidneys.

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THE EFFECT OF COOLING POWER OF THE ATMOSPHERE ON BODY METABOLISM. By J. A. CAMPBELL, D. HARGOOD-ASH and LEONARD HILL.

It has already been suggested by one of us (L. H. (1)), that basal metabolism of the body cells is raised by open-air life and exposure to cold when these conditions are not such as to produce shivering. Experiments published by others give some indication of this. Rubner(2) found the output of CO_2 per hour increased from 27·9 grms. at 23° C., to 30 grms. at 20° C., and to 32·3 grms. at 15° C. without shivering. Lusk records that the basal metabolism of dogs is lowered by confinement, and raised by a free open-air life running round a country stable. Benedict(3) reports a subject (W. W. P.) who had a basal metabolism of 2004 kilogram calories in winter and 1797 in summer, 21·4 per kilogram and 789 per sq. metre in winter, 19·2 per kg. and 707 per sq. m. in summer. This subject was an athlete, trained; his weight did not change and the temperature of the laboratory in which the observations were made was constant within a few degrees. Benedict, however, agrees with A. Loewy's(4) and J. E. Johansson's(5) conclusion that metabolism is increased by a fall of temperature only when shivering occurs. While many observers have directed attention to the influence of temperature of surroundings on metabolism, carrying out their experiments on subjects in confined places, such as laboratories and respiration chambers, the influence of the open air has been shown by observations published by one of us, L. H. (6), who gave particular attention to the cooling power of the atmosphere. In the present paper similar results are recorded, from two more subjects.

Technique. The general plan followed was constant, the morning routine being fixed for two hours preceding the experiment, which began at 11 a.m. The subject sat quietly from 11 a.m. to 11.30 a.m. reading the paper. At 11.30 a.m. the subject breathed into a Douglas bag through a mouthpiece fitted with valves. Both subjects were accustomed to the mouthpiece, having used it for several months. A 15-minute sample of expired air was usually obtained. From 11 a.m. to 11.45 a.m. numerous readings were made from wet and dry bulb thermometers, and from wet and dry kata-thermometers placed beside the subject. The cheek and

axillary temperatures and the rate of the pulse beat were recorded soon after the beginning and at the end of the experiment.

The analysis of the air and the calculations were carried out as recommended by Cathcart(7). In all cases four samples of air were taken from the Douglas bag, two being analysed by one person and two by another, with Haldane's apparatus. The average of the four results served for the calculation. The body heat production was expressed in terms of millicalories per sq. cm. per sec., this being the method of expressing heat loss as measured by the kata-thermometer. A large number of preliminary experiments of a like nature were carried out, by which every detail of technique was perfected before the experiments reported in this paper were made.

Experiments. To serve as a standard, results were obtained from the subjects sitting in a room under ordinary conditions, that is, with a dry 'kata' cooling power of about 7 millicalories per sq. cm. per sec. Numerous experiments were done on both subjects, the average heat production being about 1 millicalorie per sq. cm. per sec. and the highest 1·19 for both subjects. These figures agree very well with those usually given for basal metabolism in text-books—40 calories per sq. cm. per hour. Some results are given in the table. It will be observed that the cheek and axillary temperatures rose slightly during the experiment. This was due to the fact that the temperature of the room rose slightly, and that by sitting quiet the body heat was retained more by the clothes. In the table it will be noted that the dry 'kata' cooling powers are calculated from the temperature of the cheek, using the formula $H_2 = H_1(\theta_2/\theta_1)$ where H_2 is the dry 'kata' cooling power calculated at cheek temperature, H_1 the dry 'kata' cooling power, θ_1 is 36·5° C. minus dry bulb air temperature, and θ_2 is mean cheek temperature minus dry bulb air temperature. The 'kata' cooling power calculated at cheek temperature is with constancy four to five times that of the subject's heat production or heat loss calculated in millicalories per sq. cm. per sec., while the dry 'kata' cooling power is almost seven. It was found in previous experiments(6) that no such relation could be obtained when exposed to greenhouse conditions or warm sun, convection not sufficing for the cooling of the skin, and evaporation coming into play at very low cooling power. In the present experiments the subjects did not perspire, and were quite comfortable under a cooling power of seven.

The heat loss of the resting subject under ordinary comfortable indoor conditions—that is with a dry 'kata' cooling power of about seven—can be approximately determined by merely taking (1) the dry 'kata' cooling

power, (2) the cheek temperature, estimating the dry 'kata' cooling power at cheek temperature by means of the formula $H_2 = H_1(\theta_2/\theta_1)$ and dividing this by 4·5. In our normal subjects this result, as already stated, was about 1 millicalorie per sq. cm. per sec. It seems possible that the above formula may afford information regarding heat loss in fever patients with dry skins.

If attention be directed to the table of other experiments in various situations out-of-doors, the subjects sitting in the shade and being exposed to much higher cooling powers, it will be seen that another such relation exists between the 'kata' cooling power calculated at cheek temperature and the heat loss from the body. In this case the 'kata' cooling power calculated at cheek temperature is with constancy six to seven times that of the subject's heat production, so that in cool out-of-door conditions the heat loss in millicalories per sq. cm. per sec. of the resting subject can be calculated approximately from the formula $H_2/6{\cdot}5$. This relation has already been noted in previous experiments on other subjects(6), so that it is confirmed. A tunnel is being constructed in which experiments may be carried out under a constant cooling power. In the open air the cooling power varies somewhat from time to time. This may be responsible for the variations in the constant occasionally obtained.

One of the aims of the out-of-door experiments was to choose cooling powers which would not be likely to cause shivering. Of all the experiments here recorded only two, 14. i. 21 and 19. i. 21, gave definite evidence of shivering, the subject shivering slightly during the last two minutes of the exposure. No gloves, hat nor overcoat were worn, but otherwise winter clothes were as usual. In one or two other experiments the subject felt cold towards the end of the experiment, but there was no evidence of shivering.

Subject, C. P., appeared the more easily affected by a rise of cooling power and showed higher heat production than Miss B. under similar conditions. In most cases the cheek and axillary temperatures fell slightly out-of-doors, but the higher cooling power did not always produce the more marked fall. From these experiments it seems likely that all the body cells have their basal metabolism raised by out-of-door conditions, and that shivering is not a necessity for increased metabolism.

An experiment was done on a subject exposed to a dry 'kata' cooling power of over 20, the head, neck and hands being covered; the heat loss was not increased, under these conditions, after 40 minutes exposure. When the head, neck and hands were exposed, the heat loss rose from 0·96 to 1·44 millicalories per sq. cm. per sec., in 17 minutes. Therefore,

Date	Subject	Temperature °C. Wet bulb	Temperature °C. Dry bulb	Cheek temperature °C. Start	Cheek temperature °C. Finish	Axillary temperature ° F. Start	Axillary temperature ° F. Finish	Pulse rate Start	Pulse rate Finish	Body heat production in millicalories per sq. cm. per sec.	Kata thermometer cooling power in millicalories per sq. cm. per sec. Wet	Kata thermometer cooling power in millicalories per sq. cm. per sec. Dry (H_1)
14. iii. 21	Miss B.	11·2	16·0	29·1	30·6	96·5	97·0	66	68	0·99	18·4	6·9
16. iii. 21	,,	12·5	17·2	29·4	32·3	97·0	98·0	66	68	1·11	18·1	7·4
17. iii. 21	,,	11·5	16·6	30·9	31·7	97·0	97·0	66	68	1·01	19·1	7·2
18. i. 21	C. P.	12·0	16·5	32·2	33·1	97·0	97·2	69	68	1·18	19·1	6·7
21. iii. 21	,,	10·4	15·1	28·1	28·9	96·0	96·0	70	68	0·83	17·7	6·4
22. iii. 21	,,	12·9	17·0	32·1	31·7	98·0	97·2	80	75	1·12	16·4	6·1
24. i. 21	,,	8·0	9·8	24·0	23·9	96·6	96·2	72	66	1·29	33·4	15·8
14. ii. 21	,,	6·3	8·5	25·1	24·5	97·0	96·4	70	68	1·32	34·1	17·6
9. iii. 21	,,	7·6	9·3	23·4	24·3	97·6	97·6	73	72	1·42	37·6	17·9
19. i. 21	,,	3·0	5·0	20·3	21·9	96·8	96·0	70	66	1·46	37·1	20·3
14. i. 21	,,	0·5	2·5	21·2	21·2	97·0	97·0	68	66	1·55	36·7	21·7
7. i. 21	Miss B.	9·0	9·1	26·6	26·0	96·7	96·0	66	63	1·21	26·2	12·8
8. iii. 21	,,	5·0	6·8	21·0	21·0	97·8	97·8	68	68	1·25	37·1	23·4
6. i. 21	,,	7·1	9·0	26·0	24·0	96·0	96·0	68	68	1·31	53·0	30·0
18. iii. 21	,,	5·3	8·3	20·6	20·4	96·5	97·0	62	64	1·32	36·0	20·0
3. v. 21	C. P.	3·7	6·8	—	32·8	—	98·0	—	64	0·96	34·3	23·6
	,,	3·8	7·0	—	24·7	—	97·5	—	64	1·44	45·3	21·3

		Respiratory quotient	t_c 36·5 °C. minus air temp. dry bulb	t_s mean cheek temp. °C. minus air temp. dry bulb	H_1 Dry kata cooling power calculated at cheek temp.	H_2/H_1 Heat production	Temp. of black fur * °C.	General conditions
14. iii. 21	Miss B.	·83	20·5	13·8	4·6	4·6	17·2	In room; some windows open; low gas fire
16. iii. 21	,,	·76	19·3	13·6	5·2	4·7	17·9	,, ,, ,,
17. iii. 21	,,	·77	19·9	14·7	5·4	5·3	18·0	,, ,, ,,
18. i. 21	C. P.	·86	20·0	16·2	5·4	4·6	16·9	,, ,, no fire
21. iii. 21	,,	·80	21·4	13·4	4·0	4·8	16·8	,, ,, low gas fire
22. iii. 21	,,	·78	19·5	14·9	4·7	4·2	18·1	,, ,, ,,
24. i. 21	,,	·86	26·9	14·6	8·7	6·6	11·1	Garden; dull; cool breeze at intervals
14. ii. 21	,,	·86	28·0	16·3	10·2	7·7	11·2	Garden; fine, bright; cool breeze at intervals
9. iii. 21	,,	·85	27·2	14·5	9·5	6·7	13·0	Garden; mild, cloudy; cool breeze; few drops of rain
19. i. 21	,,	·76	31·4	15·9	10·3	6·9	6·1	Garden; bright, clear, cold wind
14. i. 21	,,	1·00	34·0	18·7	11·9	7·6	2·4	Fire escape; fine, clear, sunny, cold, bracing
7. i. 21	Miss B.	·77	27·4	16·9	7·8	6·5	9·0	Balcony; overcast, dull, misty; slight breeze, raining
8. iii. 21	,,	·80	29·7	14·0	11·0	8·8	11·0	Garden; bright, cool, no sun
6. i. 21	,,	·83	27·5	16·0	17·4	13·3	9·2	Roof; dull, bracing, very fresh breeze; chilly
18. iii. 21	,,	·76	28·2	12·2	8·6	6·5	11·0	Garden; bright, clear, cool breeze
3. v. 21	C. P.	·70	29·7	26·0	20·6	21·5	8·8	After sitting quietly on fire escape; head, neck and hands covered; dull; cold wind
	,,	·73	29·5	17·5	13·0	9·0	8·8	17 mins. after previous exp., head, neck and hands uncovered

	Age (years)	Height (cm.)	Weight (k.)
Miss B.	47	176·5	58·1
C. P.	28	161·2	52·4

* The difference between the temperature of the air and that of a piece of black fur exposed to any source of radiation indicates the influence of that source of radiant heat on the skin and clothes.

under the conditions specified the skin of the head, neck and hands is evidently responsible for the increase of heat loss. The details of this experiment are given in the table.

SUMMARY.

Further experiments are recorded showing the effects of out-of-door and indoor conditions upon metabolism. It is considered that the basal metabolism of the body cells is raised by cool out-of-door conditions, and that shivering is not necessary to raise metabolism. The metabolism is controlled by cooling power, not by temperature. Previous results are confirmed and formulæ suggested for rapid calculation of heat production of the resting subject under comfortable indoor conditions and under cool out-of-door conditions. It is considered that the former may possibly afford important information regarding patients with fever.

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EXPERIMENTS ON THE REGULATION OF THE BLOOD'S ALKALINITY. II.

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DAVIES, KENNAWAY, and the author(1) showed that in the alkalosis produced in man by the ingestion of large amounts of $NaHCO_3$ the alveolar CO_2 and the blood's CO_2 capacity rise, the urine becomes alkaline, and the ammonia excretion falls or ceases. Attempts were therefore made to produce an acidosis and study the corresponding symptoms. Up to 500 c.c. of ·116 normal HCl were drunk within 80 minutes without producing any definite fall in the alveolar CO_2, though the acid and ammonia excreted both per litre and per hour increased distinctly. Again 30 grams of $NaH_2PO_4.H_2O$ were taken without producing any fall in the alveolar CO_2, or rise in the ammonia excretion, though the titratable acid of the urine rose to ·114N. This agrees with the results of Marriott and Howland(2). Larger quantities of HCl and phosphate were not taken, as the former caused slight gastric discomfort, the latter diarrhœa.

I was led to try the effect of ingesting NH_4Cl by the observation of Palmer and Henderson(3) that the ratio of excreted ammonia to acid (titrated to phenolphthalein) remained fairly steady under various conditions. It seemed probable that this might be due to equilibrium being reached in a reversible formation of urea and acid from ammonium salts. I therefore took amounts of NH_4Cl varying from 5 to 55 grams on six different occasions. Two of these experiments lasted for a week each.

The alveolar CO_2 pressure was measured by the method of Haldane and Priestley(4), the sample being taken directly into the burette of the gas analysis apparatus from the rubber tube. Each recorded value was the mean of two samples taken at the end of inspiration and of expiration. Thus during a normal day the mean of ten consecutive pairs of half-hourly observations was 38·25 mm. The extreme values were 39·4 and 37·3 mm. and the probable error ·23 mm. or 0·6 p.c. The actual errors were however undoubtedly less than this, since the general trend of the observations showed that the true value varied slightly

during the period. Such steady results can only be obtained if the samples are taken at least 2½ hours after a meal, since digestive secretion and absorption disturb the alveolar CO_2. It is also essential that the subject should be trained to breathe regularly. Blood CO_2 capacities were determined on defibrinated blood by J. S. Haldane's(5) latest method, which is accurate within about 0·5 p.c.

The acid and ammonium salts of the urine were estimated by Malfatti's(6) method of titration with N/10 NaOH to a standard colour with phenolphthalein before and after the addition of neutralized formaldehyde solution. This method is liable to the criticisms that it overestimates the acidity and includes amino-acids with ammonia. It was however adopted for the following reasons apart from its rapidity and simplicity. The ratio of acid to ammonia in the urine only remains approximately constant if the end-point of the acid titration is at a p.H of about 9. For example a urine may contain ammonia but be alkaline to litmus or neutral red, so that if we titrated to either of these indicators the ratio would become negative. But as Davies, Haldane and Kennaway(1) showed, ammonia excretion ceases when the blood is so alkaline that large amounts (generally about ·3N) of bicarbonate are being excreted, in which case the urine gives a faint colour with phenolphthalein. Again the amino-acids may properly be included with the ammonia on the ground that in an acid urine they serve the same physiological purpose as ammonia in neutralizing acid, and must therefore be included if we wish to determine the total amount of acid excreted free and combined with non-metallic bases.

Urea was estimated by Marshall's(7) original direct titration method. The results are about 1·5 p.c. too low, but duplicates were found to agree within 0·10 p.c. Tap water may be used in place of distilled water if allowance is made for it in a blank experiment. Phosphates were estimated with uranium acetate and cochineal.

The NH_4Cl was in every case drunk dissolved in 40 times its weight of water, as any less dilution caused vomiting. This is largely a specific effect of the NH_4 ion, since 10 p.c. NaCl solution is tolerated.

The effect of 20 grams of NH_4Cl on the alveolar CO_2 is shown below. The alveolar CO_2 values in mm. Hg. taken at consecutive intervals of half-an-hour beginning at 12 noon were:

40·3	40·2*	38·9	38·0	37·8	37·6†	36·2	35·0†
35·1	34·4	34·2	34·3	34·3	34·0	34·0	33·7
(dinner)	36·2	34·8	34·9	34·4			

* Ten minutes later 10 grams NH_4Cl taken.
† Ten minutes later 5 grams taken.

On the next day the following values were obtained:

10.30 a.m.	12 noon	10 p.m.
33·2	34·5	35·9

On the third day the values were low, but within the normal range of variation.

It will be seen that the alveolar CO_2 fell fairly steadily for seven hours. The sharp rise to 36·2 after dinner was due to withdrawal of HCl from the blood for gastric digestion. The values on the following day were so low as to make it clear that the formation of acid was proceeding during the night. Hourly urines were collected on the first day of the experiment. They showed a great rise in the acid and ammonia excretions per hour, but as the volume per hour was increased fourteenfold the concentrations of both fell at first, and the ratios of acid, ammonia, and urea clearly depended as much on the water output as on anything else. It was therefore determined to study the excretions over longer periods where the volume effects would be less noticeable.

The urines were collected from midnight to noon and noon to midnight. Twenty grams of NH_4Cl were taken during the afternoons of days 2 and 3; 15 grams on the afternoon of day 4. Four meals of about the same size were taken on each day. Alveolar CO_2 samples, 80 in all, were taken at intervals, but only the averages of values at least two hours after meals are given. The results are recorded in Table I.

TABLE I.

Period	NH_4Cl taken	Alv. CO_2 in mm. Hg.	Urine in c.c.	Acid	NH_3	Acid + NH_3	Urea	Phosphate	Acid / NH_3	Ammonia N / Am. + urea N
1 A	—	38·8	1310	30·9	40·7	71·6	286	18·1	·76	·066
2 M	—	—	660	30·6	34·3	64·9	187	22·8	·89	·084
2 A	374	36·0	2040	26·5	42·6	69·1	310	15·6	·62	·064
3 M	—	—	1050	49·4	65·0	114·4	245	29·8	·77	·145
3 A	374	30·4	2840	61·6	92·9	154·5	435	25·5	·66	·096
4 M	—	—	860	58·1	95·8	143·9	231	40·7	·61	·172
4 A	281	27·8	2240	61·6	118·0	179·6	389	33·8	·52	·132
5 M	—	—	680	51·1	114·2	165·3	204	32·2	·45	·219
5 A	—	30·3	772	35·4	113·7	159·1	218	16·3	·31	·201
6 M	—	—	482	32·9	97·8	130·7	143	22·6	·35	·255
6 A	—	34·3	906	30·0	99·9	129·9	271	13·7	·30	·156
7 M	—	—	472	31·9	75·3	107·2	230	21·2	·42	·141
7 A	—	36·7	973	27·6	84·7	112·3	314	10·4	·33	·116
8 M	—	—	774	30·6	50·0	80·6	218	20·9	·61	·103
8 A	—	38·7	1310	26·7	56·5	83·2	278	7·6	·47	·092

The quantities ingested and excreted are given in c.c. of normal (in the case of phosphates, molar) solutions, i.e. milligram-molecules.

Period 3 M means the morning of the third day, and so on. It will be seen that as the result of the ammonium chloride ingestion the acid excretion per day was approximately doubled, the ammonia excretion trebled, whilst the alveolar CO_2 was reduced by 28 p.c. The lowest

alveolar CO_2's actually observed were 26·6 mm. on one occasion and 26·7 on two others.

Three samples of blood were drawn during the course of the acidosis, and two on normal days, and their CO_2 capacities determined. The results are given in Table II. In each case the blood was drawn about

TABLE II.

Time	CO_2 pressure mm.	c.c. CO_2 per 100 c.c. blood	c.c. CO_2 per 100 c.c. blood at 40 mm. pres.	CO_2 capacity as p.c. of normal	Alv. CO_2 pres. when blood drawn mm.
Normal	38·7	47·3	48·1	100	39·1
,,	36·3	46·7			38·4
3 A	41·4	33·2	32·8	68·2	31·1
4 A	36·2	27·2	28·2	58·4	27·4
6 A	43·5	44·2	42·8	89·0	33·5

4 p.m. The actual volume taken up at the pressure in col. 2 is given in col. 3. In col. 4 the volume taken up at 40 mm. pressure is calculated on the assumption that the CO_2 dissociation curve is unaltered except for the scale of its ordinates. This should be the case on Parsons' theory. The alveolar CO_2 increases with the blood's CO_2 capacity, but the two are not proportional.

When the alveolar CO_2 fell, there was very marked air-hunger. The increased frequency and depth of breathing were obvious. Thus at a time when the alveolar CO_2 was 29·7 mm. the volume breathed per minute when sitting in a chair was 10·4 litres, the normal value being about 6. Walking at three miles per hour caused severe panting, and cycling was impossible. There were occasional slight headaches. A certain exhilaration and irritability of temper were noticed at times by myself and others, but there was no mental confusion, and the experiment was not unpleasant. No increased bronchial secretion was noticed, although doses of less than 1 gram of NH_4Cl are prescribed for this purpose.

Urines examined both at the height of the acidosis and at the end of the experiment showed no albumin or sugar. There was no sign of a maximal value for the concentration of ammonia or titratable acid being reached. The highest concentration of acid recorded was ·075N, of ammonia ·193N, or ·33 p.c. The ammonia was not all excreted as chloride. Thus when the ammonia was ·193N the chloride was only ·118N. The earlier experiments seem to show that the chlorine ingested was excreted more rapidly than the ammonium.

The amount of phosphate excreted per day rose during the ingestion, since the acid excreted uncombined with ammonia is largely mono-

basic phosphate. On the other hand the phosphate excretion fell far below normal during the latter part of the experiment, even while some acidosis was still present, suggesting that a shortage of phosphate had been produced in the body during the height of the acidosis. Along with this the acid excretion fell to normal or slightly below normal, the ratio of acid to ammonia excreted being very low. That this was due to deficiency of phosphate is shown in Table III. The amounts of NH_4Cl

TABLE III.

Period	NH_4Cl taken	Phosphate taken	Alv. CO_2	Volume	Acid	NH_3	Acid + NH_3	Phosphate	Acid / NH_3
0 A	—	—	40·3	1595	28·1	33·7	61·8	21·7	·83
1 M	—	—	—	940	31·0	35·6	66·6	25·9	·87
1 A	—	—	39·3	1565	27·1	36·8	63·9	17·5	·74
2 M	—	—	—	792	30·2	32·6	62·9	23·1	·92
2 A	374	—	36·6	2005	48·5	63·2	111·7	24·1	·77
3 M	—	—	—	1325	54·3	88·4	142·7	33·8	·61
3 A	374	—	32·1	2690	54·9	100·9	155·8	28·5	·54
4 M	—	—	—	978	54·6	105·5	160·1	34·8	·52
4 A, 12–6	281	—	30·0	528	23·2	57·8	81·0	14·0	·40
4 A, 6–12	—	250	30·5	480	51·3	51·6	102·9	39·8	·99
5 M	—	—	—	765	77·3	121·4	198·7	69·2	·64
5 A	—	125	35·4	880	62·7	114·4	177·1	48·8	·55
6 M	—	—	—	715	55·1	61·2	116·3	53·2	·90
6 A	—	125	37·7	1342	42·1	65·5	107·6	40·8	·64
7 M	—	—	—	677	45·8	36·5	82·3	55·0	1·25
7 A	—	—	40·1	—	—	—	—	—	—

taken were the same as in Table II, but phosphate was taken in addition. A litre of M/2 sodium phosphate solution was made up containing 27·6 grams, or 0·2 gram molecule of $NaH_2PO_4.H_2O$, and 107·4 grams, or 0·3 gram molecule of $Na_2HPO_4.12H_2O$. This solution is feebly acid, its *p*.H being about 6·5. Half a litre was drunk between 5.30 and 9 p.m. on day 3, and 250 c.c. each on the evenings of days 4 and 5. There was slight diarrhœa, so much of this phosphate never reached the blood. Two normal days were recorded beforehand, the first being numbered 0, so that corresponding days in Tables I and III bear the same numbers. The fourth afternoon sample was divided into two portions, before and after 6.17 p.m., to show the influence of the phosphate ingestion. The alveolar CO_2's are not so accurate as those of Table I, being the averages of only 52 samples. It will be seen that on this occasion the acid excretion rose at once with the ingestion of phosphates while the ammonia excretion and the alveolar CO_2 returned to normal much more rapidly than in Table II, and the acid : NH_3 ratio never fell below ·5.

DISCUSSION.

It is clear that NH_4Cl ingestion produces a true acidosis, since the alveolar CO_2 and blood CO_2 capacity fall, and the acid excretion rises.

One of these criteria alone is not sufficient. For example pain lowers the alveolar CO_2, forced breathing lowers the blood CO_2 capacity, and acid phosphate ingestion raises the acid excretion, but none of these causes acidosis. The results obtained are exactly parallel to those of Salkowski(8) who found that NH_4Cl injected into dogs was only excreted as such to the extent of about two-thirds. In rabbits on the other hand very little was excreted unchanged, and the urine became acid. In my own case (Table II) about 13 p.c. of the NH_4Cl ingested was excreted as acid, and the ammonia must therefore have been transformed into a neutral body, and ultimately excreted in a neutral form, presumably as urea. It is quite possible that its immediate effect was to shield amino-acids from de-amination in the liver and elsewhere, as evidenced by its protein-sparing effect in starvation. This however cannot go on indefinitely, and ultimately some such reaction as:

$$2NH_4Cl + CO_2 \rightleftharpoons CON_2H_4 + H_2O + 2HCl$$

must occur. My experiments furnish no direct evidence of urea formation from ammonium chloride. In the last six days of the experiment, Table III, ·128 gram-molecule of acid, ·600 gram-molecule of NH_3, and ·470 gram-molecule of urea in excess of the normal were excreted. The diet was only roughly constant, and much of the urea excretion was obviously due to the large volumes of urine produced. The increase of ·064 gram-molecule to be expected to correspond to the extra acid was entirely masked by this cause.

The reaction postulated above is represented as reversible. Three lines of argument go to suggest that this is the case. In the first place the reaction is incomplete, in fact most of the ammonium is excreted unchanged. Now such an equilibrium is characteristic of reversible reactions only. Secondly the results of hydrogen chloride and ammonium chloride ingestions are apparently identical. Begun, Hermann and Munzer(9) found that during nine days of HCl ingestion, the average amount taken being 67·7 c.c. N acid per day, the average acid excretion was increased from 45·9 to 66·5 c.c. N per day, the ammonia from 42·2 to 80·6 c.c. N. That is to say about two-thirds of the total ingested was excreted as ammonium salt. In my own case about two-thirds was excreted in this form in the earlier part of the experiment, Table II, and although in the later part no extra acid was eliminated, the results in Table III show that this was wholly due to lack of phosphate. As however the acid ingested by Hermann per day was only equivalent to one-fifth of my average ammonium chloride ingestion, it is natural

that he never ran short of phosphate. Indeed his phosphate excretion only rose very slightly during the acid ingestion. Since, then, the reaction seems to proceed to the same equilibrium point whether we ingest hydrogen or ammonium chloride, it must be reversible. Finally, Steenbock, Nelson and Hart(10) showed that the extra ammonia excreted by calves after acid ingestion is produced entirely at the expense of urea.

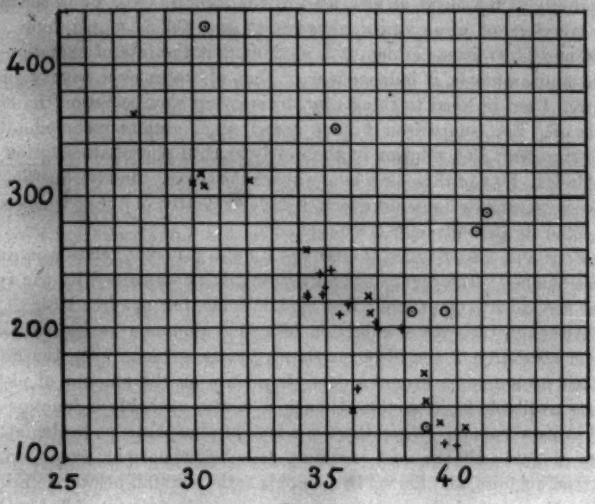

Fig. 1.

Abscissa. Alveolar CO_2 pressure in mm. Hg.
Ordinate. Millimoles acid + NH_3 excreted per day.

× Twelve and six hour urine samples.
\+ One hour urine samples.
⊙ Urine samples after phosphate ingestion.

The rates of excretion of acid alone or of ammonia alone do not depend on the degree of acidosis as shown by the alveolar CO_2, but the sum of these rates, which gives the rate at which free and neutralized acid together are excreted, seems to depend mainly on the alveolar CO_2, and very little on the rate at which water is being excreted. In Fig. 1 the rates at which acid and ammonia were excreted during 14 periods of one hour, one of six hours, and 12 of twelve hours, are plotted against

the mean alveolar CO_2 pressures during the corresponding periods. With two exceptions the points so obtained lie near a continuous curve. They can be approximately fitted by the straight line $y = 20\,(46{\cdot}5 - x)$ where x is the alveolar CO_2 pressure in mm. of mercury, y the rate of excretion of ammonia (including amino-acids) and titratable acid per day, in milligram-molecules. Whether the line is straight or not it is clear that the main factor determining the excretion rate is the alkalinity of the blood, as measured by the alveolar CO_2. Since the rate of excretion of urine varied between 44 and 497·5 c.c. per hour, or 1·06 and 11·94 litres per day, it is obvious that the rate of excretion of acid and ammonia is independent of that of water over a very wide range. I am inclined to think that during very slow secretion the rate falls off. For comparison I have added seven results recorded after drinking NaH_2PO_4 solution or the nearly neutral phosphate solution of Table III. Five of these lie a long way off the curve. Clearly superfluous dibasic phosphate ions are excreted independently of those which are required for the normal excretion of acid.

Fitz and Van Slyke(11) observed a quite different relation in diabetic acidosis. The divergence may probably be explained by the fact that the kidneys can excrete free organic acids, but not free HCl.

Although the rate of excretion of acid + ammonia varies with the under-alkalinity of the blood as shown by the alveolar CO_2, the ratio of acid to ammonia excreted depends mainly on the amount of phosphate available for excretion, though it is also probably a function of the urinary volume. When there is a lack of phosphate, as in the latter part of Table II, the acid excretion falls below normal, even during marked acidosis, and the acid : ammonia ratio may fall below one-third. When there is a plethora of phosphate as in the latter part of Table III, the ammonia excretion is relatively low, and the ratio may reach or exceed unity. It is probable therefore that very low values of this ratio, such as that of ·05 recorded by Gilliatt and Kennaway(12) in a case of pernicious vomiting of pregnancy, betoken a shortage of phosphate.

The ratio of ammonia nitrogen to total nitrogen or ammonia + urea nitrogen is often used as a criterion of acidosis. The rate of excretion of urea varies however both with the volume of the urine, as is very evident in Tables I and III, and with the amount of protein in the diet. In these experiments the ratio of ammonia nitrogen to ammonia + urea nitrogen only once exceeded 25 p.c., but a large amount of protein was being eaten, and had this not been so much higher values would doubtless have been recorded.

The absence of glycosuria, though plenty of carbohydrates were being eaten, is interesting in view of Elias'(13) observation that acid ingestion lowers the carbohydrate tolerance in dogs and rabbits. These animals had however received larger doses of acid per kilogram than the equivalents of acid ingested in my own case.

The sequence of events after ammonium chloride ingestion appears to be roughly as follows. The salt passes very rapidly into the blood, and presumably in the liver a proportion, probably a smaller part, is transformed into urea and acid which goes to lessen the alkaline reserve of the blood and other tissues. In the experiment given on p. 266 for example, the alveolar CO_2 pressure fell 1·3 mm. within 20 minutes of ingesting 10 grams of NH_4Cl. The determinations of the blood's CO_2 capacity during the acidosis, given in Table III, enable us to form a rough idea of the fate of the liberated acid. At the height of the acidosis the blood's CO_2 capacity per 100 c.c. at 40 mm. was reduced by 20 volumes. Now Davies, Haldane and Kennaway(1) showed that the blood's total combining capacity with CO_2 is about double its capacity at 40 mm. That is to say at 40 mm. pressure the alkali of the blood is about equally divided between carbonic acid and other buffers, of which hæmoglobin appears to be the most important. Hence a reduction of 20 volumes p.c. or ·009N in the CO_2 capacity at 40 mm. corresponds to a reduction of ·018N in the total alkali of the blood. Taking my blood volume at about 5 litres, the reduction of its alkali corresponds to about ·09 gram-molecule. At the time when this blood was drawn 1·03 gram-mol. of NH_4Cl had been ingested, and about ·26 gram-mol. excreted as acid or ammonia in excess of the normal. There was thus ·77 gram-mol. in the body. But only a fraction of this had been converted into acid. Had this acid been evenly distributed through the soft parts, taking their weight at 77 kgm., the decreased alkalinity would everywhere have been a fraction of ·01N, but that of the blood was decreased by ·018N, hence more than twice as much acid proportionately remained in the blood as in the other soft tissues on the average, and more than one-eighth of the whole acid remained in the blood. Had the acid been ingested directly the results would probably have been similar.

At the time when the above sample was taken the kidneys were excreting at the rate of ·36 gram-mol. of acid + ammonia per day against a normal rate of about ·13, *i.e.* ·23 gram-mol. per day extra. That is to say they were eliminating 30 p.c. of the total excess in the body per day. This contrasts unfavourably with the rate at which they can deal with excess of alkali, since in the first paper of this series it was

shown that one-seventh of the excess of alkali in the body could be eliminated in an hour, so that excess of base is eliminated 11 times as fast as excess of acid. The difference is no doubt largely due to the fact that bicarbonate can be concentrated to ·3N in the urine without increasing its p.H to more than 8·5, whilst ·3M acid phosphate solution has a p.H of less than 4, which appears to be beyond the power of the kidney to secrete.

It has been shown by L. J. Henderson(14), Hasselbalch(15), and others that the hydrogen ion concentration of plasma depends on the ratio of the concentrations of CO_2 and HCO_3. The change in the C.H. of the arterial plasma caused primarily by increasing or decreasing the CO_2 content we may call gaseous acidosis or alkalosis, that caused by decreasing or increasing the bicarbonate content non-gaseous acidosis or alkalosis. Haggard and Y. Henderson(16) showed that gaseous acidosis and alkalosis are gradually compensated by an alteration in the bicarbonate content which tends to restore the normal C.H., whilst Leathes(17), Davies, Haldane, and Kennaway(1), and others proved that this change is partly effected by the kidney.

On the other hand the lungs play the principal part in compensating the non-gaseous disturbances. The relative amounts of compensation effected by the lungs and kidneys when acid is being passed into the blood may be calculated as follows. Suppose the arterial plasma bicarbonate to be reduced by 25 p.c., then for complete compensation the alveolar CO_2 would have to be reduced in the same proportion, *i.e.* from 40 to 30 mm. This would increase the lung ventilation by about one-third. Now Campbell, Douglas, Haldane and Hobson(18) showed that an increase of about 1·5 mm. at 40 mm. in the alveolar CO_2 is required to double the normal lung ventilation. This amounts to 3·75 p.c. of the total. Hence in the case considered the alveolar CO_2 must actually be about 1·25 p.c. or ·375 mm. higher than it would otherwise be, *i.e.* 30·4 mm. instead of 30. Thus about 96 p.c. of the acidosis is compensated for by the respiratory centre, which however needs the remaining 4 p.c. as a stimulus to the increased ventilation.

I have to thank Dr J. S. Haldane for assistance with the blood-gas analyses and Dr E. F. Adolph for performing some of the phosphate estimations. Dr Adolph and Dr Kennaway have obtained similar depressions of their alveolar CO_2 from NH_4Cl ingestion, and kindly permit me to publish the fact.

SUMMARY.

1. Ingestion of ammonium chloride in man causes marked and prolonged acidosis. The symptoms include fall in the alveolar CO_2 and in the blood's CO_2 capacity, and increased excretion of acid, ammonia, and phosphates.

2. This is probably due to the conversion of part of the ammonia into urea, thus freeing the acid which had been combined with it.

3. The depression of the alveolar CO_2 below a certain value is roughly proportional to the rate of excretion of acid plus ammonia.

4. The ratio of acid to ammonia in the urine depends on the amount of phosphates available for excretion. This amount falls during acidosis.

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THE ACTION OF DIURETICS. By ARTHUR R. CUSHNY and C. G. LAMBIE.

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In the study of the action of diuretics[1], the first question to determine is whether the drug acts directly on the kidney itself or indirectly through changes in the blood or in the circulation. The changes in the renal circulation cannot be satisfactorily determined by taking the carotid blood-pressure since the local circulation is influenced by the local vasomotor mechanism; nor can it be determined by the use of an oncometer unless the secretion rate remains constant.

Direct measurement of the renal blood-flow, introduced by Barcroft and Brodie, appears to be the only method which can be employed in the study of diuresis, if we except the difficult experiments of Richards and Plant on the excised kidney maintained in apparently normal activity by perfusion. In the method of Barcroft and Brodie the renal circulation remains intact, but at intervals the renal venous blood is diverted downwards into the lower vena cava and escapes into a measuring vessel which permits the amount of blood passing in a given time to be measured. The blood is turned into the measuring pipette by placing a small clamp on the vena cava above the renal vessels and removing one below them. We have followed their method in principle, but have modified their method of collecting the blood in estimating the rate of flow. We may mention that the blood cannot be collected in an ordinary pipette for this purpose since the narrow bore offers too great resistance to the flow and it is difficult to keep the inclination of the pipette uniform in successive measurements.

The measuring cannula we have used is represented in Fig. 1. The internal diameter was 10 mm. tapering to 3–5 mm.; it had a side tube, *S*, which in use was clamped in a vertical position, and was closed at its end by the cork *C*. The cannula, apart from the side tube, usually contained 3·5 c.c.; it was paraffined inside and generally washed out with leech extract; sometimes leech extract was injected intravenously. After

[1] The work on diuresis has been discussed in detail by the senior author in his monograph on *The Secretion of the Urine*, 1917, so that it need not be given here.

an estimation had been made, the blood was blown back into the vena cava through a rubber tube attached to the side tube.

The application and removal of ordinary clamps disturbed the position of the vessels, and in addition was difficult to time exactly, and this was avoided by the use of clamps devised by Mr N. E. Condon of this

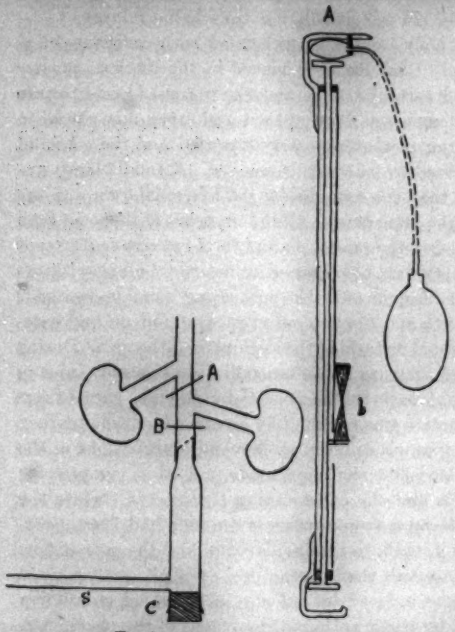

Fig. 1.

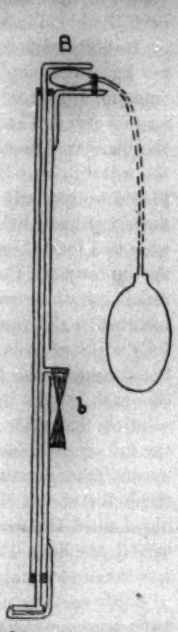

Fig. 2.

Fig. 1. Cannula for vena cava. In use the side tube *S* was fixed vertically, and not as in the figure. *A*, *B* position of the clamps on the vena cava.

Fig. 2. Condon's pneumatic clamps.

laboratory (Fig. 2). Each of these consists of a tube in which a piston is moved up and down by means of a small rubber air cushion which is inflated by pressure on a larger bulb. At the opposite end the piston carries a small plate which forms one jaw of the clamp, the other jaw being attached to the end of the tube. In *A*, inflation of the air cushion

depresses the piston and closes the clamp. In *B*, the inflation raises the piston and opens the clamp. When the air cushion is not inflated, the piston is kept in a fixed position by an indiarubber band, *b*, which raises the piston in *A* and depresses it in *B*. Both clamps were placed on the vena cava, *A* above and *B* below the entrance of the left renal vein. The time taken to divert the blood-flow, fill the cannula and drive back the blood into the vein was usually less than half a minute.

Rabbits of 2–3 kg. body weight, anæsthetised with urethane 1·5 g. per kg., were employed. Cannulæ were placed in the trachea, jugular vein (for injection) and carotid artery, and the normal blood-pressure was recorded. The abdomen was then opened and a cannula placed in the bladder. The superior mesenteric artery was tied and the whole of the intestine was ligatured *en masse* and removed. A small clamp was placed temporarily on the vena cava below the left renal vein and the aorta was ligatured at the same point. All the branches of the vena cava were tied for 2–3 cm. below the renal vein and for 1 cm. above it except the suprarenals; the suprarenal blood was thus measured along with the renal, but the error is insignificant. The measuring cannula was now inserted in the vena cava and Condon's clamps were put on and carefully adjusted so as to avoid disturbing the alignment of the vein. During the operation the blood-pressure had often fallen considerably, and in this case, it was brought back to the normal by Bayliss' gum-Ringer solution. The urine secretion was recorded by an attendant who marked the fall of each drop by an electric signal. Several observations of the normal rate of renal blood-flow were now made, one of us compressing the bulbs of the clamps and the other taking the interval before the blood filled the cannula by a stop-watch. When this had been ascertained, the drug was injected into the jugular vein and the rate of flow was taken at suitable intervals thereafter.

Caffeine. The changes induced by caffeine in the renal circulation have been investigated by many authors(1) by means of the oncometer. Landergren and Tigerstedt employed the "stromuhr" but no diuresis followed in their experiments. Schwarz measured the blood-flow directly in rabbits and found some increase in the urine under caffeine, while the blood-flow was unchanged, but the latter was so small throughout that the kidney must have been asphyxiated. In Barcroft and Straub's experiments, by the Barcroft and Brodie method, there was no definite diuresis, and the same is true of Robertson's work on dogs[1]. Richards and Plant kept the kidney alive by a special form

[1] In the dog and cat, caffeine has very little effect on the secretion of the urine com-

of perfusion, and found that caffeine may cause diuresis without any change in the blood-flow through the organ. It seemed desirable to confirm this by experiment on the living animal. After our experiments were completed, Miwa and Tamura(2) published a paper on the renal metabolism and blood-flow during caffeine diuresis, employing a method which was essentially that introduced by Barcroft and Brodie. They found that in diuresis from caffeine and its allies[1] there is no increase in the blood-flow through the kidney and no change in the O_2 taken up from the blood.

In our experiments caffeine was injected in doses of 20 mg. in about ½ c.c. of warmed Ringer or gum solution into the jugular vein. In several experiments no diuresis followed, probably owing to faulty preparation, and these have been discarded. In others diuresis set in soon after the injection. The course of the experiment may be followed from the tracings in Figs. 3 and 4. In these the injection of caffeine was followed by a sharp fall in the blood-pressure which rose again to the normal height or beyond it in about one minute. During the return of the blood-pressure or immediately after it, there followed a transient acceleration of the renal blood-flow. In Fig. 3 this acceleration was from 6 seconds to 5·1 secs. at the maximum, or 15 p.c., while in Fig. 4 the time required to fill the cannula fell from 4 secs. to 3·1 secs., an acceleration of 22·5 p.c. This acceleration lasted 4–5 minutes, and the rate of flow then returned to the normal or became slower. The flow of urine began to increase about one minute after the injection, as the blood-pressure was recovering and rather before there was any definite dilatation of the renal vessels; this is well seen in Fig. 4 in which we succeeded in measuring the rate of flow between these two points. The rate of secretion increased for 4–5 minutes and then slowly declined, reaching the normal in about 15–20 minutes after the injection.

The fall in blood-pressure has been noted by many observers and is generally attributed to direct action on the heart muscle; it lasts a very short time and may be followed by a slight rise above the normal, which may probably be due to the vasoconstriction in compensation for the previous fall in pressure.

The acceleration of the blood-flow through the kidney occurs as the blood-pressure rises or after it has completely recovered, and the two features are quite distinct in time. It seems not unlikely that they have

pared with that in the rabbit. Only one of Barcroft and Straub's experiments was done on the rabbit and here there was very little effect.

[1] Diuretin increases the O_2 absorption owing to its content of salicylate.

a similar origin, however, and that the action on the heart muscle which reduces the blood-pressure is followed by depression of the vascular wall which leads to dilatation and an acceleration of the current. In both

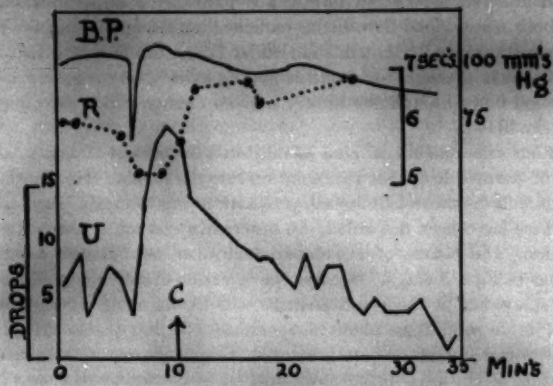

Fig. 3.

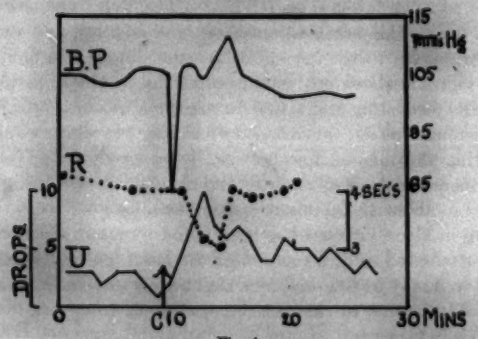

Fig. 4.

Figs. 3 and 4. Blood-pressure, *B.P.*; number of seconds required to fill the measuring cannula, *R*; drops of urine per minute, *U*; Caffeine injection (0·02 grm.), *C*.

cases the muscle is weakened by direct action of the caffeine in high concentration, but as soon as the concentration in the blood falls, the muscle recovers; the heart muscle is affected sooner than the renal

vessels, and the short fall in blood-pressure therefore precedes the acceleration in blood-flow. The dilatation of vessels when caffeine is perfused through them is a well-known phenomenon and has led to the use of caffeine as a vasodilator in disease of the coronary arteries; it does not seem to have been realised how very short this effect is in the living animal.

This acceleration of the blood-flow appears at first sight to give an adequate explanation of the diuresis. But in the experiment from which Fig. 4 is drawn, the increase in the urine began rather before the accelerated flow, and in all cases the diuresis continued long after the blood-flow had returned to the normal rate or was even slower. Unless the short dilatation of the vessels be conceived to have a prolonged effect on the rate of secretion, this seems to be fatal to the idea that the increased blood-flow and diuresis under caffeine are correlated. In several experiments a second injection of 0·02 grm. caffeine was made after the first diuresis had passed off. This was generally followed by the same transient changes in the blood-pressure and renal blood-flow, but the urine generally ceased and in no case was it increased. The poisonous action of caffeine on the kidney was apparently induced by the second dose.

Miwa and Tamura observed no change in the renal blood-flow under caffeine during diuresis, but this is easily explained by the fact that they never took the rate of flow until 10–15 minutes after the injection. At this stage the blood-flow has long returned to the normal, while the diuresis may persist. We thus agree with them that the caffeine diuresis is independent of the rate of blood-flow through the kidney, and must arise from a direct action on the kidney cells.

Pituitary extract. The diuresis from the injection of extract of the posterior lobe of the pituitary body has been examined by means of the oncometer by Schäfer and others, who found that the kidney volume generally increases while the blood-pressure rises from the constriction of the vessels in other regions; but the diuresis may occur without any change in the blood-pressure or in the volume of the kidney. Robertson estimated the outflow in the renal vein and found no increased blood supply under pituitary extract.

In our experiments we used Duncan and Flockhart's "pituitary fluid" generally in doses of 0·2 c.c. The intravenous injection was followed in every instance by a considerable acceleration in the rate of flow through the kidney and by a rise in the general blood-pressure (Fig. 5). These changes persisted for 15–25 minutes. The blood-pressure

reached its highest point rather sooner than the maximum acceleration as a general rule, and both then slowly fell to the normal. The diuresis was small compared with that from caffeine, while the acceleration of the blood-flow was more marked and persistent. There was in some experiments a short period of 3–4 minutes after the injection during which the secretion was smaller than before the injection or ceased altogether. After this the urine increased in amount and then slowly fell off again, reaching the normal point about the same time as the return of the blood-pressure to its former level. In a number of experiments under pituitary the change in the blood-pressure and rate of flow was unaccompanied

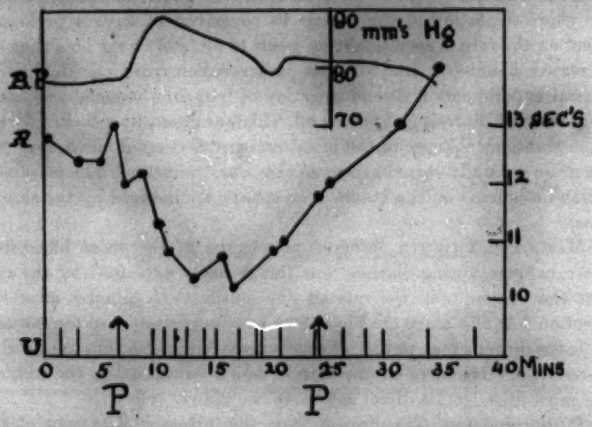

Fig. 5. Blood-pressure, *B.P.*; number of seconds required to fill the measuring cannula, *R*; drops of urine recorded on the base line, *U*. Injection of pituitary fluid 0·2 c.c., *P.P.*

by any significant change in the amount of urine secreted. But when diuresis was induced by pituitary, its course ran so nearly parallel with the blood-flow through the kidney, that it suggests that diuresis is the result of the larger supply of blood to the kidney and not of any action on the secretory cells.

Strophanthin. The effects of the digitalis series in increasing the urine, an early clinical observation, have been examined repeatedly by means of the oncometer, but not by any more direct method of determining the renal blood supply. In our experiments a specimen of crystalline strophanthin prepared by Sir Thomas Fraser from Strophanthus

glaber was used in doses of 0·01–0·02 mg. This was sufficient to cause a slight increase in the urine of a rabbit in which cannulæ had been placed in the jugular vein and bladder, without further operative procedure. But in a series of five experiments in which the animal was prepared as described above for the measurement of the rate of blood-flow through the kidney, strophanthin in these doses had no diuretic effect and gave no appreciable change in the blood supply. Larger quantities increased the blood-pressure and reduced the flow through the kidney, probably from a direct constricting action on the renal vessels. Normal animals often show no diuresis after the administration of members of the digitalis series, and even in successful experiments the increase in the urine is small compared with that from caffeine and its allies. The severity of the operation may also have contributed to the absence of effect in our experiments, and especially the exclusion of the large intestinal area. It is still unproved that the diuresis in cases of dropsy arises from changes in the renal circulation under this series, for it may be argued that the improved nutrition of other tissues leads to the reabsorption of effused fluid into the blood, and that the consequent hydræmia is the efficient factor in the increased secretion of urine. This could be disproved only by the measurement of the plasma percentage in case of dropsy before and after digitalis treatment. If the diuresis arises indirectly from the absorption of fluid, hydræmia would be present, while if it is the result of changes in the renal circulation, digitalis ought to reduce the fluid of the blood. Whether the change in the plasma attains dimensions measureable by our present methods may be regarded as questionable.

Sodium sulphate. Barcroft and Brodie, and Barcroft and Straub found that the renal blood-flow was sometimes increased under saline diuresis, but that this is transient when present and is not proportional to the urine. This was confirmed by Lamy and Mayer, who further pointed out that the diuresis was accompanied by acceleration only when large quantities of salt solution were injected, while smaller quantities increased the urine without change in the blood-flow. Robertson observed acceleration of the flow during diuresis, while Richards and Plant state that salt solution may cause diuresis without any change in the blood supply to the kidney.

We have measured the blood-flow under the injection of sodium sulphate in a number of animals, generally at the end of experiments in which other diuretics had been injected. The solution was 20 p.c. and 5 c.c. were injected. Marked diuresis followed at once and the rate of flow through the kidney rose, but soon returned to the normal, while the

urine was increased for long afterwards. Our results thus confirm those of the previous workers—that the diuresis is independent of the increased blood supply to the kidney. One of us has already pointed out that the efficient factors in the saline diuresis are the dilution of the colloids of the blood, and the osmotic resistance to the reabsorption of the filtrate in the tubules owing to the salts themselves being absorbed to only a limited extent.

Urea. The injection of urea (1 grm. in 2 c.c. Ringer solution) was followed in two experiments by diuresis with acceleration of the blood-flow, but the latter was short-lived, while the urine remained high after the normal rate of blood-flow had returned (Fig. 6). The urea diuresis is thus not dependent on the blood-flow only, but on the same factors as have been mentioned under sulphate diuresis.

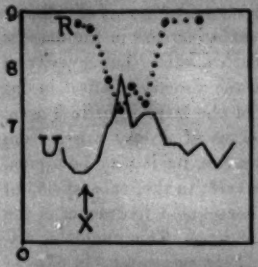

Fig. 6. Number of seconds required to fill measuring cannula, *R*; drops of urine per minute, *U*; injection of urea 1·0 grm., *X*.

Gum solution. In several experiments, injections of Bayliss' 6 p.c. solution of acacia in warm Ringer were made, generally when the blood-pressure had fallen greatly and the flow through the kidney was slow. In some of these, the blood-pressure rose to the normal, the blood-flow was quickened and the urine increased. In other experiments in which the blood-pressure was satisfactory before the gum was injected, there was a smaller rise in blood-pressure, the rate of flow was accelerated, but no significant change in the urine followed. The difference in these results may perhaps be explained by the mechanical effects of the gum solution: when the blood-pressure is low from the exhaustion of the animal, the urine ceases from the failure of the pressure in the glomerular capillaries and the gum in raising the pressure restores the secretion, even though it does not itself supply a readily filtrable fluid. On the other hand, when the blood-pressure and renal circulation are normal, this mechanical action in increasing the bulk of the blood and raising the blood-pressure by a slowly diffusing fluid has comparatively little effect on the amount of urine. The gum solution increases the filtrate by improving the blood-pressure in the glomerulus, not by diluting the colloids; the salts act by diluting the colloids without necessarily changing the blood-pressure or the renal circulation time.

Adrenaline. Several experiments were performed with adrenaline 0·05 c.c. 0·1 p.c. solution rather as a test of the method than with the idea of ascertaining any new facts. Fig. 7 gives the blood-pressure, and the measurement of the rate of blood-flow and of the urine has been inserted beneath. The parallelism between the two upper curves is remarkable: the urine practically ceased during the high pressure and returned as it passed off.

Section of the splanchnic nerve. In two experiments the left splanchnic nerve was cut in the course of the experiment. This was followed by a short slowing in the rate of the renal blood-flow, and after 1–2 minutes by acceleration. Unfortunately no record of the secretion was made in these two experiments, but many writers have found it increased by

Fig. 7. Blood-pressure tracing, *B.P.*, ×½; time required to fill measuring cannula, *R.* Drops of urine recorded on *U*. Time in 10 seconds. *A*, injection of adrenaline.

splanchnic section; the increased blood-flow has also been recorded by Burton-Opitz and Lucas (1908) by means of a "stromuhr."

Discussion and Summary.

Among the diuretics examined, the increase in the urine appeared to depend directly on the blood-flow through the kidney in only one instance—the pituitary extract. Here the diuresis set in with the acceleration in the venous flow and passed off as it disappeared, and the action may thus be attributed to the improved circulation in the kidney; according to the Heidenhain school this may act by improving the nutrition in the organ, while others would ascribe the increase in the urine to a higher pressure in the glomerular capillaries and to a more rapid replacement of the plasma; the concentration of the colloids by the drainage of the fluid through the glomerular filter is thus counteracted through the more rapid replacement of the blood.

Under caffeine the diuresis begins before the accelerated flow and lasts long after it has become normal. The action is thus not dependent on changes in the blood supply but on changes in the renal cells. This has been assumed to be the action by a number of investigators, who have generally located it in the epithelium of the tubules. But the chief effect of the purine diuretics is by common consent an increase in the water of the urine, while the solids are relatively slightly augmented. This seems to point to an action on the glomerular capsule rather than on the tubules, whatever view be held of the division of labour among the renal elements. The simplest view which reconciles the observations is that caffeine causes diuresis by reducing the resistance to filtration through the glomerular capsule by a specific action on its cells.

The diuresis from salts and urea is not necessarily accompanied by acceleration of the renal blood-flow, though this may accompany it in the beginning, and presumably favours its occurrence. One of the chief factors is the hydræmia, which reduces the osmotic resistance to filtration by diluting the colloids, the other the difficulty of reabsorption of these bodies in their course through the tubules.

Adrenaline lessens the blood supply and thus the secretion of urine, while section of the splanchnic nerve has the opposite effect in increasing the blood-flow through the kidney and the amount of urine.

Strophanthin had no diuretic action in our experiments, nor did it alter the circulation through the kidney in moderate doses, while larger ones increased the blood-pressure, slowed the renal blood-flow and lessened the secretion of urine.

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THE COAGULATION OF THE BLOOD. Part II. The Clotting Complex.

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In a previous paper(1) it was shown that the calcium of the blood is normally present in two forms, ionised and combined, and that it is the combined calcium which is essential for the inauguration of normal blood-clotting. The term clotting complex was applied to the combination of calcium with an undefined organic material. The present paper gives an account of an investigation indicating the organic substance to which the calcium is attached, and of an attempt to prepare artificially a compound with coagulating powers and general properties resembling those of thrombokinase.

Anticoagulants. The action of certain anticoagulant substances used in the subsequent work was first considered. Anticoagulants may be divided into two groups, salts and animal derivatives. The salts may again be divided into two groups: those which act mechanically owing to their concentration, as in the various types of salted plasma where clotting is re-established by proper dilution, and those which, uniting with the calcium of the clotting complex, necessitate the addition of ionised calcium in order to restore coagulation. Into the latter group fall the oxalates, citrates and fluorides whose action has already been discussed; they act essentially as antikinase. The anticoagulants of animal origin most commonly used are hirudin, cobra venom and peptone; the first two of these are able to inhibit clotting *in vitro* or *in vivo*, but peptone has no action *in vitro* and causes a temporary incoagulability when injected intravenously.

The method of calcium estimation previously described affords a means of testing the action of the last group of anticoagulants. Coagulation may be arrested at one of two stages; either at the stage of activation of thrombokinase, or the activity of the formed thrombin may be destroyed. In the first case, the amount of ionised calcium present should be 6–7 mg. p.c., since clotting has not truly commenced; the action of the anticoagulant would then be that of an antikinase. In the second case where thrombin is formed but is inactivated, the amount of ionised calcium present should be the same as that found after clotting has occurred, *i.e.* 10–11 mg. p.c. This argument is based on the accepted theory that

calcium plays no part in the composition of thrombin, but only in the changes preceding its formation. Table I shows the results of a series of calcium estimations in blood treated with these anticoagulants. The technique was the same in each case; blood and anticoagulant were mixed, with the latter in minimal amount to obtain the inhibitory effect; the mixture was incubated for 15 minutes at 37° C., heated for an hour at 55° C. and the calcium estimated.

TABLE I.

Anti-coagulant	Ionised Ca. mg. p.c.	Anti-coagulant	Ionised Ca. mg. p.c.
Hirudin	6·35	Cobra venom	6·82
	6·66		6·59
	6·60		6·74

Hirudin and cobra venom therefore act primarily as antikinase. It is stated that hirudin acts also as an antithrombin; in order to test this the following experiment was carried out. A thrombin solution was prepared which readily caused clotting in blood to which 100 mg. p.c. of ammonium oxalate had been added. Constant volumes of thrombin solution were incubated at 37° C. for ten minutes with varying strengths of hirudin, and the oxalated blood then added. Cobra venom (denoted in future as C.V.) was similarly tested.

Exp. 1. Oxalate conc. 100 mg. p.c. Thrombin sol. 4 vols.

	Hirudin				Cobra venom		
37° C. mins.	1/3000	1/4000	1/5000	37° C. mins.	1/1000	1/2000	1/3000
6	0	0	0	6	0	0	*a*
8	0	0	*a*	8	*a*	*a*	*b*
10	0	0	*b*	10	*c*	*c*	*d*
12	0	0	*d*	12	*d*	*d*	*d*
15	0	*a*	*d*				

The extent of coagulation is marked here and throughout as 0, no clot; *a*, slight clot; *b*, marked clot; *c*, nearly complete clot; *d*, complete clot. These experiments show clearly that hirudin has an inhibitory action on thrombin, whereas venom has practically none; these results are in accordance with the work of Mellanby (2). Hirudin, therefore, appears to act on that factor of the clotting mechanism which is in greater concentration, kinase or thrombin, whereas cobra venom is purely an antikinase.

The clotting complex. Some reference may be made to certain biochemical results obtained in connection with coagulation. One of the most important factors in the clotting of shed blood is the blood-platelets. It is generally held that they are one of the chief sources of thrombokinase. It is stated that by gross chemical analysis the platelets contain

nucleoprotein, lecithin and cholesterin(3). Many workers have suggested that the lipoids play an important part in blood-clotting. Wooldridge(4) in 1883 put forward evidence indicating that lecithin was closely connected with coagulation. Stuber, Partsch and Heim(5, 6) showed that the addition of lipase to extracted sera restored the clotting power, that the activity of thrombokinase was due to its ether-soluble fraction, and that the coagulative power of organ extracts was due to the fats and lipase. Kephalin, a lipoid which is widely distributed throughout the blood and tissues, has been found to have active coagulant power, and it has been suggested that the kephalin fraction of the lipoids is responsible for coagulation. A fact of considerable significance is that kephalin is rarely completely free from calcium(7). Of the two most common lipoids, lecithin and kephalin, the close association of the latter with calcium suggests that it may have the more powerful action; further, kephalin is more soluble in saline solutions, and unlike lecithin does not tend to be precipitated from its solutions by divalent or monovalent ions.

Howell(8) found that dried organs, such as the brain and thymus, contained an ether-soluble phosphatide which he considered to be kephalin, and which possessed marked clotting activity. In saline and aqueous tissue extracts the phosphatide was combined with a protein which had a low temperature of coagulation, 60° C. The phosphatide in combination with the protein was precipitated by boiling, but was not harmed by boiling by itself. Aqueous solutions of the phosphatide were found to be precipitated by the addition of neutral salts or of salts of the alkaline earths, and finally the thromboplastic activity of these solutions deteriorated on standing. These are some of the reactions of the substance which Howell terms thromboplastin. The same author(9) found that his prothrombin could be precipitated from oxalate blood by acetone, and that it was activated to form thrombin by calcium salts without the addition of any third substance. These reactions all point to the probable identity of the material investigated with a lipoid compound, and later work will show a very close resemblance between Howell's substances and artificial mixtures of calcium and a lipoid.

There is, therefore, some evidence that three substances may play a part in the formation of the clotting complex, a protein, a lipoid and calcium: these indications were followed in the subsequent work.

Nucleoprotein was prepared from the pancreas of a bullock by the method of extraction of the minced gland with 0·8 p.c. NaCl and precipitation with acetic acid; a solution of this protein had no coagulant power *in vitro*. A suspension of lecithin was made from the commercial material,

and a solution of calcium chloride was used as a basis. On mixing these substances a solution was obtained which possessed coagulant power; in this preliminary work the components were not used in measured amounts. With this solution the following reactions were obtained, and compared with a fresh saline extract of rabbit's testis.

Exp. 2. Anticoagulant. K.F. = 800 mg. per 100 c.c. blood.
Blood 10 vols. Lecithin sol. 2 vols. Complete clot in 6 mins.
" Testis extr. 2 vols. " "

Controls showed the amount of calcium added in the solution did not cause any clotting in six minutes in the fluoride blood; the calcium alone was not therefore responsible for the clotting. In order to avoid any excess of calcium being present in ionised form, the lecithin solution was shaken up with an equal volume of 1/800 oxalate solution, thus reducing the concentration of its constituents by half and precipitating any ionic calcium; the solution was then re-tested.

Exp. 3. Anticoagulant. K.F. = 800 mg. per 100 c.c. blood.
Blood 10 vols.
Lecithin sol. 2 vols. Complete clot in 6 mins.
Testis extr. 2 vols. " "

These preliminary experiments indicate that the added lecithin has marked coagulant power in the presence of calcium.

Solutions were next used having known concentrations of their constituents; for convenience of reference the formulæ of the mixtures are given, and each represented by a number. The volumes of reagents used were constant; 10 vols. of blood were prevented from clotting by the addition of 2 vols. of anticoagulant, and to this 2 vols. of coagulant mixture were added. The solutions were made in distilled water.

Exp. 4. Sol. 1. N-protein 0·25 mg. p.c. Sol. 2. N-protein 0·167 mg. p.c.
Lecithin 0·25 " Lecithin 0·167 "
Sol. 3. Lecithin 0·25 " $CaCl_2$ 0·167 "
$CaCl_2$ 0·25 "

Hirudin 1/3000.

37° C. mins.	Sol. 1	Sol. 2	Sol. 3
4	0	0	0
6	0	*a*	*a*
8	*a*	*b*	*b*
10	*a*	*d*	*d*

There is thus seen to be increased activity of the solutions in which calcium is present; the clotting shown in sol. 1 is almost certainly due to the combination of the added lecithin with the ionised calcium of the blood. It was observed that soon after making sol. 3 the lecithin was precipitated by the calcium added, but this did not happen in sol. 2 for

a much longer time and then more slowly. The protein may act as a buffer between the calcium ions and the lecithin, and the protective action may be due to the adsorption of the lecithin by the protein molecule.

Two fresh solutions were made up containing the same substances in much reduced amounts, the calcium concentration being approximately equal to that of the total blood calcium.

Exp. 5. Sol. 4. N-protein 0·01 mg. p.c. Sol. 5. Lecithin 0·01 mg. p.c.
Lecithin 0·01 „ $CaCl_2$ 0·03 „
$CaCl_2$ 0·03 „
Hirudin 1/3000

	Sol. 4				Sol. 5			
37° C. mins.	1/1	1/10	1/100	1/1000	1/1	1/10	1/100	Control
4	0	0	0	0	*a*	0	0	0
6	*d*	*c*	*a*	0	*d*	*a*	0	0
8	*d*	*d*	*b*	*a*	*d*	*b*	0	0
10	*d*	*d*	*d*	*b*	*d*	*c*	0	0
12	*d*	*d*	*d*	*d*	*d*	*d*	0	0

This shows that the solution containing nucleoprotein is more active than the one where it is absent, again emphasising the apparent protective action of the protein. The conclusion so far suggested by these experiments is that the lipoid is the substance most active in coagulation when combined with calcium, but that a protein may also be necessary as a secondary factor, making the solution more sensitive, perhaps by preventing the precipitation of the lecithin by the divalent calcium ion.

It has been shown previously that the clotting complex of normal blood can adsorb oxalate in excess of the chemical equivalent of its calcium content. Experiments were carried out with the object of ascertaining the identity of the component which was able to effect the adsorption referred to. Oxalate solutions of varying concentration were added to the solutions 4 and 5, and also to a solution of lecithin alone. The ammonium oxalate and lecithin-containing solutions were mixed and incubated at 37° C. for 15 minutes, and the blood then added to them; no hirudin was used.

TABLE II.

Oxalate conc.; mg. per 100 c.c. blood	Degree of clotting in 10 mins. at 37° C.		
	Sol. 4	Sol. 5	0·1 p.c. lecithin
50·0	*d*	*d*	*d*
57·1	*d*	*d*	0
66·7	*d*	*d*	0
72·7	*a*	*a*	0
80·0	0	0	0

The blood used was normally prevented from clotting by the addition of 57·1 mg. of oxalate per 100 c.c. of blood. The above results show that

lecithin has no coagulative power in the absence of ionised calcium. The calcium content of the solutions 4 and 5 corresponds to an addition of 1·82 mg. p.c. of calcium to the blood, and this is equivalent roughly to 5 mg. p.c. of oxalate; clotting should therefore be inhibited at an oxalate concentration between 57·1 and 66·7 mg. in Table II. The conclusion may be drawn from these results that lecithin, in the presence of oxalate, can only cause clotting when it is combined with calcium; it can then adsorb oxalate in excess of the chemical equivalent of the calcium attached to it.

A similar experiment was carried out using hirudin instead of oxalate. Varying concentrations of hirudin and a constant volume of the coagulant solutions were mixed and incubated at 37° C. for ten minutes; blood containing 57·1 mg. p.c. of oxalate was then added, and the mixture returned to the incubator for ten minutes; the degree of clotting was then noted. 1·09 mg. per 100 of blood was then added to each tube and the whole incubated for a further period of ten minutes; the final readings were then taken.

Exp. 6. I. Oxalate conc.; 57·1 mg. p.c. blood.

Hirudin dil.	1/3000	1/2000
Lecithin 0·5 p.c. ...	0	0
Sol. 4	*d*	0
Sol. 5	*d*	0
Calcium 1·09 mg. p.c.	0	0

II. 1·09 mg. Ca per 100 c.c. blood added.

Hirudin dil.	1/3000	1/2000	1/1000	1/500
Lecithin 0·5 p.c. ...	*d*	*d*	*d*	*d*
Lecithin 0·005 p.c.	*d*	—	—	0
Sol. 4	*d*	*d*	*d*	*b*
Sol. 5	*d*	*d*	*d*	0

It has been shown that 1/3000 hirudin prevents the clotting of normal blood so that the clotting must here be due to the neutralisation of the hirudin by one of the added substances. The first part of the experiment shows that lecithin even in large amount is unable to overcome the action of the hirudin in the absence of calcium, and that calcium itself is also unable to cause clotting in the absence of the added lecithin. The solutions which alone contain both calcium and lecithin, solutions 4 and 5, are able to cause clotting at a hirudin concentration of 1/3000 but not at 1/2000. In the second part of this experiment there is excess of ionised calcium present, and the results obtained indicate that the degree of coagulation is roughly proportional to the amount of lecithin contained in the added solutions. It is therefore evident that lecithin is able to cause clotting in the presence of hirudin only when it is associated with ionised

calcium. The same results were obtained on substituting cobra venom for hirudin.

Other properties of these solutions were examined. The calcium lecithin compound was found to be relatively heat stable; its activity after heating was tested against hirudin as follows:

Exp. 7. Hirudin 1/3000.

37° C. mins.	Raised to 100° C.		100° C. for ½ hour	
	Sol. 4	Sol. 5	Sol. 4	Sol. 5
4	0	0	0	0
6	*a*	*a*	0	*a*
8	*b*	*b*	0	*b*
10	*d*	*d*	0	*d*

This experiment shows that the substance is in itself heat stable, but when it is in the presence of a protein, heating at 100° C. for half-an-hour causes precipitation of the protein and of the lecithin with it. The temperature at which activity is lost depends on the temperature of coagulation of the protein used.

Solutions of this calcium-lecithin compound lose power in a manner similar to thrombokinase solutions when they are kept. The activity of the solution used was taken as that dilution at which complete clotting occurred in ten minutes at 37° C. in the presence of 1/3000 hirudin, all volumes being constant.

TABLE III.

Days	Active strength	
	Sol. 4	Sol. 5
0	nearly 1/1000	1/50
2	1/100	1/50
6	1/50	1/50
9	1/10	1/10
21	coagulant power lost in both solns.	

The properties of sol. 4 may thus be summarised as follows:

1. The activity of the solution depends on an ether-soluble lipoid substance in suspension, in combination with calcium.
2. The activity of the calcium-lipoid compound is increased by the presence of a protein.
3. The compound is able to coagulate oxalate, hirudin or cobra venom blood.
4. The solution is not affected by raising the temperature to 100° C. It loses its activity on prolonged boiling, and deteriorates on keeping.
5. The calcium-lipoid compound is precipitated by acetone; the precipitate is unchanged in its properties.

There is therefore a resemblance between this artificial mixture and normal kinase, so far as the properties of the latter are known.

The calcium-lecithin compound was next prepared in bulk. 500 c.c. of a 1 p.c. solution of partially purified lecithin were treated with an excess of a concentrated solution of calcium chloride, so that the lecithin was completely precipitated. The precipitate was filtered off, washed with distilled water and examined. In suspension in water this substance was found to have properties similar to those summarised in the case of sol. 4, and in addition it was found to be capable of causing clotting in fowl's plasma, which had been prepared in such a way that it would only clot on the addition of solutions containing thrombokinase.

The lecithin so far used was not of a high degree of purity. After further purification repetition of some of these experiments gave similar results, the chief difference being that there now appeared to be a greater activity of the calcium-lecithin solutions used. No attempt was made to separate the lecithin and kephalin fractions of the material used.

There seems therefore to be evidence that the necessary factor in the early stages of blood clotting, is a combination of calcium and a lipoid. The substance so formed resembles the activated thrombokinase of Morawitz rather than the prothrombin of Howell. In the latter case, since the combination of prothrombin and calcium produces thrombin, if the calcium-lipoid compound were of this class it should give the reactions of thrombin. It does not do so, however, since it is not able to cause clotting in the presence of a large excess of oxalate, and its action is inhibited by cobra venom which has no apparent action on thrombin. On the other hand the work recorded seems to cast doubt on the identity of thrombokinase with a ferment. It is not at present possible to say whether the combination of calcium and lecithin is present as such in the circulating blood, or whether when blood is shed, the ensuing physical changes cause the union to occur.

Summary.

1. The inauguration of blood coagulation depends on the presence of a complex in which three substances are combined; these are a lipoid, calcium, and a protein.

2. The lipoid and the calcium are essential. The calcium-lipoid combination does not yield calcium ions.

3. The function of the protein is apparently secondary; it seems to render the lipoid compound more sensitive, and may act by keeping the latter in a state of fine suspension.

4. The compound described has properties resembling those of the thrombokinase of Morawitz rather than of the prothrombin of Howell.

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THE EFFECTS OF RESPIRATION OF OXYGEN ON BREATHING AND CIRCULATION.

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It was shown by Paul Bert(1) that exposure of living organisms to an abnormally increased partial pressure of oxygen has, in general, a detrimental effect. In the case of warm-blooded animals, however, the effect which he discovered was only shown at oxygen pressures exceeding about three atmospheres. At pressures considerably higher than this the central nervous system was soon damaged irreparably, so that the animals did not recover. When removed from the atmosphere they remained comatose, and displayed symptoms similar in many respects to those of strychnine poisoning, and resembling also the after-effects of prolonged exposure to want of oxygen, as in CO poisoning. His description of the spastic condition of the limbs and easily induced opisthotonus in the animals experimented on corresponds rather strikingly with the similar symptoms recorded by one of us in miners who had been rescued alive after prolonged unconsciousness from CO produced in colliery explosions(2).

Lorrain Smith(3) discovered that though oxygen at less than three atmospheres' pressure produces in warm-blooded animals no evident nervous symptoms, yet prolonged exposure to even as little as 70 p.c. of an atmosphere of oxygen (or 60 p.c. in the alveolar air) may produce fatal pneumonia. The lung epithelium is of course exposed to the full effects of the oxygen; and it can hardly be doubted that if the far more sensitive tissues of the central nervous system were really exposed to a considerably increased oxygen-pressure very evident nervous symptoms would be produced. As will be shown in a paper shortly to be published by Douglas and Haldane, the blood passing through the brain of man loses under normal conditions probably not more than about 2 c.c. of oxygen per 100 c.c. of blood, and the CO_2-pressure of the blood does not rise more than about 3 or 4 mm. The co-efficient of absorption of oxygen in blood at body-temperature is ·022. Hence when pure oxygen is breathed at two atmospheres pressure there will be in simple solution in the arterial blood, allowing for the presence of about 6 p.c. of

$CO_2 + H_2O$ in the alveolar air, about 4·14 c.c. of oxygen per 100 c.c., or 3·9 c.c. in excess of what is normally present. Hence if the circulation rate remained normal, there would be about 2 c.c. of free oxygen per 100 c.c. of the venous blood returning from the brain, and the oxygen-pressure in the brain would therefore be extremely high.

The experiments now to be described were undertaken for the purpose of seeing whether any evidence could be obtained that the tissues of the nervous system are defended against the influence of the oxygen by diminution of the circulation through them, so that the excess of free oxygen in the arterial blood is used up as it passes through the capillaries, and thus cannot, except at extremely high pressures of oxygen, reach the tissues. The diminution in the circulation through the brain capillaries might easily be brought about by contraction of capillaries in the manner recently described by Krogh(4). If the circulation is diminished when oxygen is breathed it is evident that the pressure of CO_2 in the tissues must rise; and in the respiratory centre this rise of CO_2-pressure will, other things being equal, imply rise of hydrogen ion concentration and consequent increase in breathing and fall of alveolar CO_2-pressure. A very slight fall in alveolar CO_2-pressure will, however, suffice to compensate for the rise in CO_2-pressure in the tissues in consequence of a sufficient slowing down of the circulation to reduce the oxygen-pressure of the venous blood to normal. Complete compensation could not, however, be expected, since otherwise there would be no stimulus left to account for the slowing down of circulation.

The problem which we set ourselves to investigate, therefore, was whether there is any fall in alveolar CO_2-pressure when oxygen at increased partial pressure is breathed. We also watched the pulse carefully, as any diminution in pulse-rate would serve as an index of slowing of the circulation. It was already known from the careful experiments of Parkinson(5), that an appreciable, though small, diminution in pulse-rate is produced by breathing pure oxygen. Most of our experiments were carried out at ordinary atmospheric pressure; but one series was at increased atmospheric pressure in the large steel chamber of the Lister Institute. We are much indebted to Professor C. J. Martin for personal help and facilities afforded to us for this series.

The experiments were made as follows: two large Douglas bags were filled respectively with pure oxygen and air. The subject then sat quietly and breathed from one of the bags through Rosling valves and a comfortable mouthpiece (with a nose-clip on the nose) for at least five minutes. Towards the end of this period the pulse was repeatedly counted, and

finally a sample of alveolar air, obtained about one second after the completion of an inspiration, was taken by the ordinary Haldane-Priestley method, and the CO_2 determined.

The mean results at ordinary atmospheric pressure were as follows:

	Alveolar CO_2 percentage		Pulse		Number of pairs of observations
	oxygen	air	oxygen	air	
L. D.	6·04	6·25	75·1	81·0	36
J. S. H.	5·25	5·42	78·0	82·7	3

The following results were obtained in a very careful series made on one day in the steel chamber at a total pressure of 2·08 atmospheres or 1580 mm.

	Alveolar CO_2 percentage		Pulse		Number of pairs of observations
	oxygen	air	oxygen	air	
L. D.	2·46	2·69	76·5	87·7	4

If we convert CO_2 percentages into pressures of CO_2 the results are as follows:

		Alveolar CO_2-pressure in mm. of Hg.		
		Oxygen	Air	Fall with oxygen
At ordinary atmospheric pressure ...	L. D.	42·8	44·3	1·5
	J. S. H.	37·2	38·4	1·2
At 2·08 atmospheres	L. D.	37·7	41·2	3·5

Out of the 43 pairs of observations the CO_2-pressure was only in six cases lower with air than with oxygen; and only in four cases was the pulse-rate higher with oxygen.

It is clear from these observations that when oxygen is breathed at normal barometric pressure there is a drop of about 1·5 mm. in alveolar CO_2-pressure, and about five beats per minute in the pulse during rest sitting. At 2·08 atmospheres the drop is about 3·5 mm. in alveolar CO_2-pressure, and eleven beats per minute. The experiments therefore confirm the theory (which is in itself probable from many considerations into which we need not enter here) that excess of free oxygen in the arterial blood causes slowing of the circulation. The result of this slowing down will be that in accordance with the conception running through so much of Claude Bernard's work, the "internal environment" of the tissues tends to be kept approximately constant in spite of great variations in the external environment. We can thus understand why it is that so high a pressure of oxygen is needed before the central nervous system is affected. The observed drop of 3·5 mm. in the alveolar CO_2-pressure when oxygen at 2·08 atmospheres pressure was breathed would

correspond to a slowing in the brain circulation sufficient to reduce the oxygen-pressure in the blood leaving the brain to normal. An oxygen-pressure such as we tested in the steel chamber is sufficient to cause fatal pneumonia within two or three hours. Neither of us, however, could detect any definite subjective symptoms of psychical disturbance on breathing the oxygen for a few minutes. On the other hand distinct symptoms of irritation of the air-passages were produced in L. D., and did not pass off for several hours.

Our attention was directed to a paper by Yamada(6) in which his alveolar CO_2-pressure was carefully measured in order to investigate the effects of breathing mixtures of oxygen, air, and CO_2. These experiments showed that whether or not CO_2 was present in the inspired air the alveolar CO_2-pressure was distinctly lower with oxygen than with air. He concluded that the oxygen increased the excitability of the respiratory centre to CO_2. This is, of course, a possible explanation, but is not probable in view of the evidence that want of oxygen increases the excitability of the centre to CO_2, or contributes towards exciting it(8). In any case the experimental results of Yamada are in complete harmony with our own.

Conclusion.

Respiration of oxygen, particularly at increased barometric pressure, increases the breathing and diminishes the pulse-rate. These effects are presumably brought about by slowing of the blood-flow through the tissues, which protects them against the poisonous action of the high oxygen-pressure.

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THE COMPARATIVE ATROPHY OF THE SKELETAL MUSCLE AFTER CUTTING THE NERVE AND AFTER CUTTING THE TENDON.

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Quantitative knowledge on the course of atrophy of muscle under different conditions, is still imperfect, though some observations in conditions other than those of simple nerve section have lately been made by Langley and his co-workers(1).

We have made a step towards filling in the data by comparing the course of atrophy after cutting the nerve and after cutting the tendon of skeletal muscles. On rabbits the sciatic nerve of one side in its course between the muscles of the thigh was cut and a piece of about 1 cm. was excised. At different intervals after the operation the m. gastrocnemius, soleus and plantaris of the normal and the operated side were excised, weighed and dried. In other experiments the tendon of the same muscles (tendo Achillis) was cut and a piece of 1 cm. was excised; the fresh and the dry weights of the muscles of the normal and of the operated side were compared at different intervals after the operation. The error in these experiments does not exceed about 6 or 7 p.c. In nine experiments performed on normal animals the average difference between the right and the left muscles was − 0·5 p.c. for the fresh and − 0·9 for the dry weight, the maximal difference being − 6·3 and − 4·2 p.c.

The results of the two series of experiments are given in the following tables.

It will be seen that in these experiments up to 40 p.c. of the dry weight of the muscles was lost in about two weeks, more than 50 p.c. in four weeks, and that only about 10 p.c. more was lost in a further 60 days. It will also be seen that the course of atrophy was the same after cutting the nerve and after cutting the tendon. This is especially clear if we form "curves of atrophy" with the percentage loss of dry weight as ordinates and the interva!s after the operation as abscissæ (Fig. 1). We obtain similar curves if we use the original minimal and maximal instead of the average figures. Similar curves are obtained also on using the figures for the percentage loss of fresh weight.

TABLE I. ANIMALS WITH NERVE SECTION.

Number of Exps.	6	10	5	4	3	3
Duration in days	6	14	28	56	84	123
Average fresh weight of the muscles, grm.:						
(*a*) normal side	11·17	8·93	10·27	9·86	8·41	7·71
(*b*) operated side	10·43	6·81	6·35	4·68	3·56	2·70
Average percentage difference	−6·6	−23·7	−38·2	−52·5	−57·7	−65·0
Average dry weight of the muscles, grm.:						
(*a*) normal side	2·61	2·09	2·40	2·18	1·95	1·67
(*b*) operated side	2·36	1·42	1·29	0·93	0·86	0·55
Average percentage difference	−9·8	31·9	−46·1	−57·7	−55·9	−67·1
Minimal and maximal percentage differences:						
(*a*) for the fresh weight, min.	− 3·8	− 8·7	−16·2	−34·7	−33·1	−62·4
„ „ max.	−10·9	−35·4	−52·1	−61·0	−63·9	−68·5
(*b*) for the dry weight, min.	− 8·0	−16·8	−30·2	−45·1	−29·3	−57·2
„ „ max.	−11·5	−40·8	−55·9	−65·2	−67·1	−75·3

TABLE II. ANIMALS WITH TENDON SECTION.

Number of Exps.	6	16	5	4	5	3
Duration in days	6	12–16	28	56	84	123
Average fresh weight of the muscles, grm.:						
(*a*) normal side	11·06	8·34	9·61	9·73	12·11	8·69
(*b*) operated side	11·09	6·69	5·73	4·90	5·32	3·77
Average percentage difference	+ 0·4	−19·9	−40·5	−49·6	−56·1	−56·6
Average dry weight of the muscles, grm.:						
(*a*) normal side	2·63	1·94	2·21	2·24	2·76	2·00
(*b*) operated side	2·45	1·37	1·19	1·08	1·28	0·88
Average percentage difference	− 7·0	−29·0	−46·1	−51·9	−53·7	−55·9
Minimal and maximal percentage differences:						
(*a*) for the fresh weight min.	+ 6·1*	− 0·1*	−28·9	−46·3	−41·5	−52·7
„ „ max.	− 7·1	−41·8	−45·6	−52·5	−65·6	−62·1
(*b*) for the dry weight min.	− 0·6	−12·8	−36·3	−48·3	−42·1	−50·5
„ „ max.	−17·7	−41·6	−51·2	−54·7	−68·9	−63·1

The average p.c. difference is calculated from the weights to three places of decimals, in the Table the fresh and dry weights are only given to two places.

* These minimal figures are caused, as it will be seen further, by the greater water content of the atrophying muscle.

In experiments of this nature there is a possible error, *i.e.* that the loss of weight of the muscles on the operated side is caused to a certain degree by an increase of weight of the muscles on the normal side. But this possible error could not have been of any importance in our experiments, since the weight of the animals after operation varied very little. As a further test, Audova tried the effect of loss of body weight by

keeping for one to two weeks without food, rabbits in which the tendo Achillis had been cut on one side. He found that, whilst the muscles in the later period were about 50 p.c. less than normal in weight, the difference in weight on the two sides was the same as in the earlier experiments.

How can one explain the loss of weight the muscle undergoes after its tendon was cut? From the experiments of Heidenhain(2) and of Fick(3) we know, that the heat production of the muscle decreases when the load is smaller. When the tendon is cut we have a similar condition of the muscle. The work done is decreased. Thus the loss of weight after

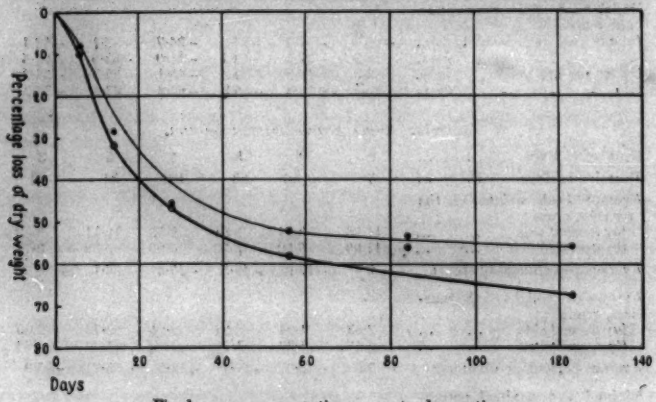

Fig. 1. —— nerve section; —— tendon section.

cutting the tendon would seem to be a pure case of atrophy by decreased activity. There are two possible objections against this point of view:

It might be said that the loss of weight after cutting the tendon is caused, not by decreased activity but by the operation itself setting up some pathological condition. This question was examined by a series of experiments in which the tendon was exposed, but instead of excising a piece a longitudinal incision was made in it. These experiments showed that in the first 14 days after the operation there may be some loss of weight; but this loss of weight was found only in three out of ten experiments, whereas in all experiments in which a piece of the tendon was excised there was, 14 days after the operation, a very noticeable loss of dry weight, cf. Table II. Further, four experiments on tendon incision

showed, 53 days after the operation, no loss of weight. We see that if the longitudinal incision of the tendon can cause a loss of weight, this must be the result of some pathological condition acting in the beginning. But this condition evidently ceases afterwards and cannot be detected in the longer experiments.

Another objection which might be made is that a transverse incision, or an excision of a piece, of the tendon severs afferent nerve fibres and thus suppresses some centripetal impulses which normally influence the work and the condition of the skeletal muscle. As there is no experimental evidence on this question it cannot be said whether this objection is justified or not.

If we put aside the latter objection we can say that there is an identity in the course of atrophy caused by cutting the nerve and by decreasing the activity of the muscle by cutting the tendon. It seems that inactivity plays a great rôle in the atrophy after injury of the nerve. It is true that the course of atrophy after cutting the nerve is quicker than after cutting the tendon; but this can be explained by the assumption that in the first case there is an absolute inactivity, whereas in the second case there is only a decreased activity, the muscle still receiving impulses from the central nervous system and contracting without load.

When the muscle undergoes atrophy there is not only a loss of fresh or dry weight, but also a change in the chemical composition. As already stated Audova found that the relative amount of water in the muscle after cutting the nerve and after cutting the tendon is distinctly greater than in the normal; Audova and Takson stated further that the relative amount of water soluble substances is also increased if atrophy takes place. An augmentation of the relative amount of water in the organs has been found also by different authors in case of inanition(4) and in inanition Lipschütz(5) found an increase of the relative amount of water soluble substances. It seems that these water soluble substances are products of decomposition of the organic constituents of the living cell, and that the increase of their relative amount and the increase of the relative amount of water take place in all cases when there is an increased decomposition of living matter.

Our assumption that inactivity plays a great rôle in the mechanism of the atrophy of the skeletal muscle after the nerve was cut is in contradiction to the view put forward by Langley(1).

Langley has contested the theory that the atrophy of muscle is due to absence of contraction, since he found that making the muscles contract by electrical stimulation after their nerves had been cut did not

delay the atrophy. An absence of effect of stimulation has also been found by Hartmann and Blatz(6). However this may be, the experiments recorded in this paper show that a decrease in the work performed causes atrophy, and we think that this can only be explained on the theory that atrophy is due to inactivity.

Langley does not come to any definite conclusion as to the cause of atrophy, but he suggests that it may be due to over-use caused by the continuous fibrillation. It is known that fibrillation may continue for a long time, we have seen it four months after nerve section. Langley and Hashimoto (*op. cit.* p. 64) state that there is a broad correspondence between the degree of fibrillation and degree of atrophy, but that there is insufficient evidence to decide whether the fibrillation is the cause of the atrophy or merely an accompanying phenomenon. It does not seem to us probable that even continuous fibrillation represents a sufficient expenditure of energy to cause a loss of weight. The increase in oxygen consumption in atrophying muscle found by Langley and Itagaki, which they think favours the theory of over-use, can be explained as due to the oxidation of waste products formed in the decomposition of inactive atrophying muscle.

SUMMARY.

Section of the tendo Achillis in rabbits causes atrophy of muscle which is nearly as great as that caused by nerve section. The atrophy is caused by the great decrease of work performed, *i.e.* by inactivity.

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THE REGULATION OF THE EXCRETION OF WATER BY THE KIDNEYS.

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THE starting point of the present work was the well known fact that great diuresis follows the ingestion of large amounts of water *per os*, and the attempt has been made to find out whether this diuresis is related to any changes in the blood comparable to the changes in hydrogen ion concentration which regulate the breathing.

In a paper published in 1915(1) it was shown by Dr J. S. Haldane and myself that as a consequence of drinking water the excretion of urine could be increased from about 40 c.c. per hour up to as much as 1200 c.c. per hour, the specific gravity falling to less than 1001. Comparative estimations of the amount of hæmoglobin in the blood before and during diuresis did not however show any appreciable change, though the method of estimation of hæmoglobin used was capable of giving results accurate to about 1 p.c. In another paper(2) it was shown, however, that the above-mentioned diuresis was accompanied by a distinct change in the electrical conductivity of the blood serum, amounting to about 5 p.c., and the conclusion was drawn that the water absorbed from the alimentary canal did not remain in the blood stream but was distributed in the tissues and that at the same time salts passed out of the blood into the tissues, or the water in the alimentary canal, to an appreciable extent.

On resuming this work the observations as to the production of diuresis by water drinking and the constancy of the percentage of hæmoglobin in the blood were confirmed.

Fig. 1 shows a composite curve giving the average results of 15 experiments in several of which hæmoglobin estimations were made. None of these showed any definite change in relation to the diuresis. Nevertheless, it seemed likely that some relation existed between the diuresis and the composition of the blood. Now Haldane(3) has shown meanwhile that, in order to make theory agree with the experimental facts as to the relations between temperature, pressure and volume in gases, it is necessary to introduce some modifications into the equation expressing

the behaviour of gases under conditions of varying temperature and pressure, and that so modified this equation permits quite simply of the extension of the gas laws to liquids. Moreover, it follows that "osmotic pressure" is not due to bombardment of a semipermeable membrane by molecules of a solute and therefore proportional to the concentration of the solute in unit volume of solution. On the contrary, osmotic pressure is simply the increased external pressure necessary to neutralise the greater diffusion pressure of water molecules in pure water or a weak solution when separated from a more concentrated solution by a semi-

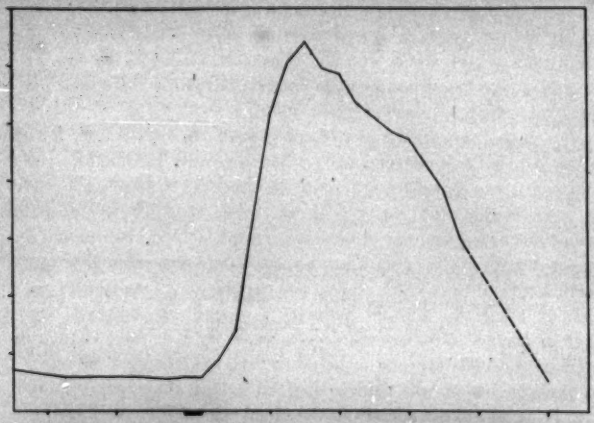

Fig. 1. Ordinates = excretion of urine per hour. Each division represents 100 c.c. Abscissæ = time in hours. During period marked by black line two litres distilled water drunk.

permeable membrane. The diffusion pressures of water on the two sides are proportional to the respective ratios between water molecules and total molecules. What is ordinarily referred to as the "osmotic pressure" of aqueous solutions, such as blood, which are not under any increased external pressure, is simply the excess in the diffusion pressure of water molecules in pure water over the diffusion pressure of water molecules in the solution. At any given temperature the difference between the (aqueous) vapour pressures of pure water and of the solution is exactly proportional to the differences of diffusion pressure of water in the two liquids.

This new conception of the extension to liquids of the kinetic theory of gases makes it possible to form an intelligible theory of the probable connection between the rate of excretion of water by the kidneys and the so-called "osmotic pressure" of the blood. On Van't Hoff's theory that osmotic pressure is in some way due to the combined pressure exercised by the solute molecules, the reason why excretion of water should depend on the osmotic pressure of the blood is quite unintelligible. Since it appeared probable that, if there is a physical factor which correlates the composition of the blood with the activity of the kidneys, this factor is likely to be the diffusion pressure of water in the blood, attention was directed to the possibilities of estimating the diffusion pressure of water in the blood. Attempts to prepare a semipermeable membrane, by means of which measurements of the osmotic pressure of the blood before and after drinking could be made, were without success; and no changes in vapour pressure of water could be detected. No appreciable change in size of red corpuscles after drinking water was demonstrated by means of the hæmatocrit; nor, using Scarpa's method, were any changes evident in the viscosity of the serum. My experiments have therefore been made on the water and chloride content of the blood.

Estimation of the loss in weight on drying blood. In several experiments, which gave concordant results, it was found that there is after drinking a slight but distinct increase in the relative amount of water contained in blood as estimated by the loss of weight on drying. Blood was withdrawn from a vein by means of a syringe and three samples of about 1 grm. each were introduced into tared weighing bottles containing glass wool. These bottles were at once weighed against similar counterpoise bottles to 0·1 mgrm. and were then placed in a desiccator containing phosphoric anhydride. Two litres of water were then drunk and when diuresis had set in and was judged to be at about its maximum a second sample of blood was taken and treated as above. All six bottles were kept in the desiccator until of constant weight. One experiment may be quoted as an example of the results obtained:

Blood taken before water drinking		Blood taken after drinking	
Sample (1) lost on drying	77·45 % of its weight	Sample (1) lost on drying	77·99 % of its weight
„ (2) „	77·44 „ „	„ (2) „	77·94 „ „
„ (3) „	77·43 „ „	„ (3) „	77·86 „ „
Average = 77·44 ± 0·006		Average = 77·93 ± 0·038	

It appears therefore that during the diuresis produced by water drinking, the blood is more dilute than before to the extent of about 2·3 p.c., *i.e.* 100 grm. of blood taken after drinking contain about 0·5 grm.

more water than the same blood taken before drinking. This result agrees satisfactorily with both the hæmoglobin and conductivity estimations previously made and with the chloride observations set out below, provided that the relative increase of water in the blood after drinking is due to passage of solids out of the blood.

Estimations of the chloride content of the blood. Several experiments were carried out to ascertain whether the chlorides of the blood showed any appreciable alteration after water drinking. For this purpose the method devised by Van Slyke(4) was employed. Whole blood, not plasma, was used in order to avoid any possible introduction of error through interchange of ions between plasma and corpuscles. It was found that control titrations rarely differed among themselves by more than 0·05 c.c. KI solution = 0·005 p.c. NaCl.

The experiments were always carried out in the same manner, viz. at 8.0–8.15 a.m. breakfast was taken, including coffee, and not earlier than 11.0 a.m. a sample of blood was removed from a vein for chloride analysis. About 6 to 8 c.c. of blood were withdrawn from a vein by means of a syringe containing just enough potassium oxalate to hinder coagulation. Of the blood so obtained, two samples of 2 c.c. each were taken for chloride analysis, thus providing four filtrates for the final titration. No food, drink or exercise was taken through the day, and about 4 p.m. a second sample of blood was taken and treated as above. The same pipette was used for measuring the blood in each case and the necessary measuring flasks were carefully calibrated by weighing.

On the following day or shortly afterwards the above procedure was repeated with the exception that after the first sample of blood had been taken two litres of distilled water were drunk and one or more samples of blood were taken during the ensuing diuresis. The urine was measured at suitable intervals and its chloride content estimated. Fig. 2 gives the results of one such experiment.

It will be seen that on the control day the blood chloride at noon was just above 0·48 p.c. and at 5 p.m. it was just below 0·48 p.c. On the water drinking day the blood chloride, starting at 0·49 p.c., fell during diuresis to 0·455 p.c. and rose again to 0·47 p.c. as diuresis passed off. Meanwhile the percentage of chloride in the urine (calculated as NaCl) fell from 1·34 to 0·08. The hourly excretion of chloride fell steadily from 1·18 grm. to 0·09 grm. per hour with the exception of a rise from 0·42 to 0·64 grm. per hour at the onset of diuresis. This rise, however, only lasted a short time and the normal fall was soon resumed in spite of the continuance of the diuresis. In this experiment the maximum diuresis amounted to about

520 c.c. per hour and was attained about 1½ hours after drinking. On the control day the percentage of chloride in the urine fell from 1·3 p.c. to 0·36 p.c. and the hourly excretion from 1·36 grm. to 0·33 grm. per hour.

The results of this experiment agree well with the conductivity estimations recorded previously and it is clear that the diminution of conductivity and blood chloride after water drinking cannot be accounted for entirely

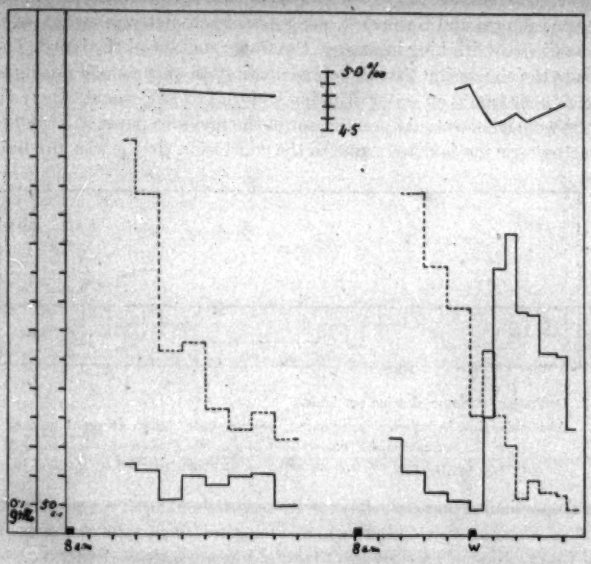

Fig. 2. Ordinates. Continuous line = excretion of urine per hour. Interrupted line = excretion of chloride per hour.

Abscissæ = time in hours. At 8 a.m. breakfast. At *W* two litres water drunk. Upper continuous line = blood chloride.

by dilution of the blood by the water ingested. This, as shown by the hæmoglobin determinations and also by the loss of weight on drying, amounts to less than 2·3 p.c., while the fall in conductivity and blood chloride amounts to about 5 to 6 p.c. It therefore appears to be clear that the water absorbed is only taken up in the blood to a slight extent, *i.e.* about 25 to 30 grm., and that the remainder is stored elsewhere along with the salts and probably albumin which have passed out from the

blood. This result agrees with the observations of Engels(5), who found that on injecting normal saline into the vessels of dogs the excess of liquid retained in the body over that excreted in the urine etc. was laid up mainly in the muscles and skin. The injection lasted one hour and the animals were killed three hours later. The water content of the blood was then increased 1·92 p.c., of the skin 3·87 p.c. and of the muscles 3·86 p.c., *i.e.* the blood retained 11 grm. of water, the skin 126 grm. and the muscles 482 grm. Engel and Scharl(6), using the refractometer, investigated the effects of water drinking in man on the water content of the blood. They came to the conclusion that there was no dilution of the blood during the diuresis consequent on water drinking and that Strauss and Chajes'(7) results were incorrect. As pointed out in the previous paper(2) I tried the refractometric method and came to the conclusion that it was unreliable

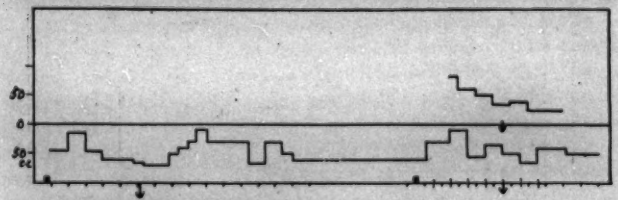

Fig. 3. Ordinates = volume of urine per hour.
Abscissæ = time in hours. Upper curve, 100 c.c. water taken at point marked by arrow. Lower curves, 100 c.c. water + 15 gm. NaCl taken at points marked by arrows. Second day 100 c.c. water taken at points marked by vertical lines.

for this particular purpose. Blix(8) has estimated the dry residue of the blood of rabbits before and after introducing water into the stomach. He used Bang's torsion balance and found a dilution of from 1 to 2 p.c., but his results are remarkable in that the maximum dilution of the blood did not appear until from 4½ to 7½ hours after the introduction of the water into the stomach of the animal.

Excretion of water and chloride in the urine during water diuresis. In an ordinary experimental day in which breakfast, including a cup of coffee, was taken about 8 a.m. and subsequently no food or drink till after the completion of the observations it was found that the excretion of urine fell within a couple of hours of breakfast to about 50 c.c. per hour and usually remained fairly uniformly about this figure. The excretion of chloride however fell progressively during the day (cf. Fig. 2). If after some hours a dose of 15 grm. sodium chloride in 75–100 c.c. of water was

taken the excretion of urine per hour rose gradually from 70 to 90 c.c. and then slowly fell. A control experiment in which 100 c.c. distilled water was drunk in place of the solution of 15 grm. NaCl in 100 c.c. water showed only a slight interruption in the descent of the curve. Fig. 3 shows the results of these experiments.

As mentioned in the description of Fig. 2, the great increase in the volume of urine excreted in consequence of drinking two litres of water is usually accompanied by a transitory increase in the absolute amount of chloride excreted per hour. This "washing out" of chlorides however only lasts a very short time and the normal fall of chloride excretion is

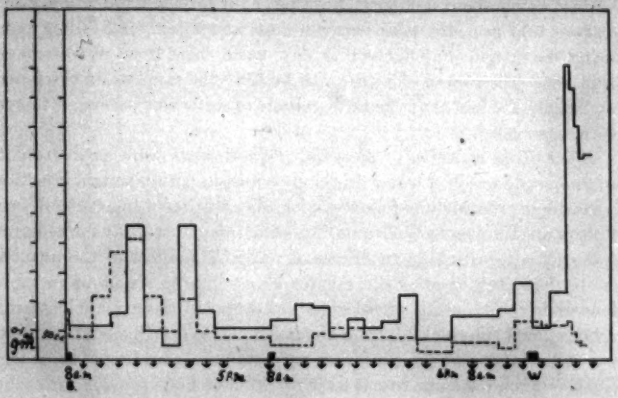

Fig. 4. Ordinates. Plain line—hourly excretion of urine. Interrupted line—hourly excretion of chloride.

Abscissæ = time in hours. At *B*, breakfast of toast and coffee. At hours marked by arrows, 100 c.c. water and two biscuits taken. At *W*, three litres water drunk.

usually resumed at a time when the water diuresis is still increasing. If the store of chloride in the body has been diminished by a chloride poor diet so that the hourly excretion of chlorides is low the water diuresis may be obtained undiminished without any increase in the excretion of chlorides. Fig. 4 shows the results of such an experiment (last three days).

For three days the diet consisted of two pieces of toast and half a cup of coffee for breakfast and thereafter two biscuits (containing ·005 grm. chlorine) and 100 c.c. water hourly till 5.30 p.m. At 7.30 dinner was taken consisting of rice pudding and one baked apple with some cream and sugar

and also half a pint of whisky and soda. At 11 p.m. another glass of whisky and soda was taken. On the fourth and fifth days the toast and coffee for breakfast were replaced by two biscuits and 100 c.c. of water. On the fifth day between 11.10 and 11.40 a.m. three litres of water were taken of which 350 c.c. were vomited at 11.40, the remainder being retained. During the first three days the excretion of both water and chloride showed considerable irregularities, probably largely due to the effect of the coffee taken for breakfast. During the last two days the irregularities in the excretion of water were very much diminished and the excretion of chloride showed a slight rise during the middle hours of the day, being about 0·06 grm. NaCl per hour for the night urine, rising to about 0·11 grm. per hour between noon and 2 p.m. and falling again during the afternoon. On the fifth day, when three litres of water were drunk, the excretion of chlorides was sensibly the same as on the fourth day despite the fact that the total volume of urine was increased to over 500 c.c. per hour.

Effect of the injection of pituitrin. Experiments were carried out to determine the result of water drinking combined with pituitrin injection. The ordinary routine was followed with the addition of injection of 1 c.c. of pituitrin (Burroughs Wellcome, infundin) intramuscularly immediately or shortly after drinking two litres of water. The effect of the pituitrin was to delay the onset of diuresis for about four to six hours or more. Meanwhile the excretion of chlorides and water in the urine fell steadily and the blood chlorides fell from about 0·5 p.c. to 0·47 p.c. and remained low. After four to six hours diuresis set in and the excretion of urine then followed much the same course as it would have done some hours earlier if no pituitrin had been injected. In experiments where the excretion of chloride was followed during the onset of diuresis it was found that the chloride diuresis tended to run parallel with the water diuresis, unlike the course of excretion of chlorides in simple undelayed diuresis following water drinking without injection of pituitrin. Fig. 5 shows the results of a pituitrin experiment (compare with Fig. 2).

In one experiment where the pituitrin was not injected until 25 minutes after the water was drunk a slight diuresis began, but was quickly cut short and a delay of five hours followed before the diuresis again became evident. During the initial diuresis the excretion of chlorides was considerably increased, but then fell steadily till the main diuresis set in when it again increased and tended to run parallel with the excretion of water. A few determinations of blood-pressure were made and they showed a slight increase after injection of pituitrin, *e.g.* before injection 120 mm. Hg.

and two hours after 130 mm. Hg. Experiments with phenol red showed that the time of appearance of this substance in the urine was unaffected by injection of pituitrin.

Discussion of Results.

The observations recorded in this paper show that there are slight but definite changes in the composition of the blood accompanying the

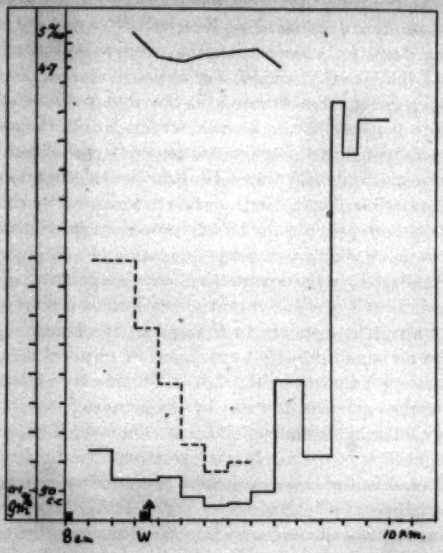

Fig. 5. Ordinates. Plain line—excretion of urine per hour. Interrupted line—excretion of chloride per hour.

Abscissæ = time in hours. At *W*, two litres water drunk. At †, 1 c.c. pituitrin injected. Upper line = blood chloride.

diuresis provoked by drinking water. These changes and others previously recorded, *i.e.* diminution of electrical conductivity, diminution of blood chlorides and dilution as shown by weight of residue after drying, are of such a nature that the diffusion pressure of water in the blood at the time of the diuresis must be definitely increased. In fact it appears that a tenfold increase in the activity of the kidneys as regards excretion of water is correlated with diminution in "osmotic pressure" of the blood plasma

from about 1775 to 1700 mm. Hg. or with a corresponding increase of diffusion pressure of water (cf. Haldane(3)). This increase is about 4½ p.c. as compared with an increase of at least 1000 p.c. in the secretion of urine.

Numerous attempts (*e.g.* those of v. Korányi(9)) have been made to deduce some law connecting the activity of the kidneys with the "osmotic pressure" of the blood on the one hand and that of the urine on the other. These attempts have not been attended by much success, nor is this surprising. At the time they were made the views of osmotic pressure universally held were dominated by Van't Hoff's tentative conception that osmotic pressure is proportional to concentration of solute in a solution, and the fact that this theory is not even accurately in agreement with experimental observation in the case of dilute solutions and altogether out of agreement in the case of stronger solutions, was slurred over or generally ignored. Again the experimental difficulties of the enquiry are considerable and it would appear from the figures given above that, if the excretion of water in the urine is regulated by changes in the diffusion pressure of water in the blood, these changes will be very small. In fact the state of affairs as regards regulation of excretion of water is apparently analogous to the conditions affecting regulation of the respiration, and as it is well recognised that alterations in the lung ventilation are brought about in response to changes in the blood which are too minute to be measured directly by chemical or physical means, it is not unnatural to expect that a similar state of affairs may prevail as regards regulation of the excretion of water by the kidneys.

Moreover, a further complication arises. For, even if one assumes that the main regulating factor as regards excretion of water is the diffusion pressure of water in the blood, one must also admit that to some extent the excretion of water is influenced by the excretion of other substances. Thus Ambard(10) has shown that the urinary constituents are largely but not entirely independent as regards their excretion, and he devotes considerable space to a discussion of what he terms "concentration maxima." He finds in fact that such a substance as urea is excreted quite independently of say chloride, but that the kidney is only capable of concentrating urea in the urine up to a certain point. If the excretion thus attained is inadequate to remove urea at a rate commensurate with the needs of the body the amount of water excreted also increases. Addis and Watanabe(11) also find that the addition of urea to a diet with constant water intake causes considerable increase in the volume of urine excreted. Results of a similar nature were obtained by Adolph(12) in a recent paper. He found that a dose of sodium chloride increased the rate of excretion

of water. This increased loss of water cannot be due to any reaction of the kidneys to increased diffusion pressure of water in the blood. Adolph also found however that, if he took a large dose of sodium chloride during the diuresis caused by drinking water, the rate of excretion of urine was at once greatly diminished.

My experiments (cf. Fig. 3) confirm these results of Adolph in both respects, though in my experiments the diuresis produced by sodium chloride seems to be less pronounced.

In seeking for an explanation of the fact that, while the excretion of water by the kidneys is apparently regulated by the diffusion pressure of water in the blood, there are also conditions in which increased excretion of water is caused by other factors, it is necessary not to lose sight of the fact that the renal epithelium is in contact not only with the blood on one side but also with the urine on the other. Water is not the only substance the excretion of which is regulated, and in conditions where there is great concentration of the urine while at the same time the stimulus to excretion of some solid constituent is urgent, it may easily happen that water is so to speak dragged out along with the solid. Otherwise, this solid constituent might reach such a concentration in the urine as to oppose an insuperable resistance to further excretion, owing to the low diffusion pressure of water in the urine compared with that in the blood.

Figs. 3 and 4, however, show that the excretion of water is regulated to a large extent independently of that of chloride. Nevertheless the effect of "concentration maxima" of other substances on the excretion of water cannot be ignored.

Since the results shown in Fig. 4 apparently indicate the essential independence of the excretion of water and of other substances in the urine except for the effect of conditions where "concentration maxima" comes into play, it would seem that the transitory rise in the excretion of chlorides when water diuresis sets in, which is seen in the results of other experiments, is due to the fact that the greater dilution of the urine facilitates the excretion of chloride. The chloride content of the blood is normally above the threshold; hence the increased excretion when this process is assisted by the effects of water diuresis. The excretion of chloride, however, soon falls again because the diminishing content of chloride in the blood acts in the opposite sense to and outweighs the effects of dilution of the urine. In other words the lack of immediate adaptation is probably due to the activity of the kidney as regards excretion of water being controlled by a two-fold regulation as suggested above, viz. a main regula-

tion due to diffusion pressure of water in the blood and a subsidiary modification of this regulation by the conditions existing where Ambard's "concentration maxima" comes into play.

These conclusions receive support from the work of Bock and Iversen(13) who found that during water diuresis there was no increased excretion of phosphate and that the proportion of phosphate in the blood remained constant. During theophyllin diuresis on the other hand, the phosphates increased in the urine and diminished in the plasma. Marshall(14) finds that during water diuresis the excretion of water may increase twenty-fold while that of creatinine is unaltered, that of urea is increased slightly, *i.e.* not more than twice, while the chloride excretion is variable. The chloride excretion is often increased but never so much as urea and moreover the excretion of chloride decreases during maximum diuresis.

Several observers in recent years have drawn attention to the fact that injection of pituitrin often leads to inhibition of the excretion of urine. On the other hand there are numerous accounts of the diuretic effect of pituitrin injections. In my experiments the probability seems to be against the inhibitory effect being due to diminished absorption for diarrhœa was not provoked as one would expect to be the case if large quantities of water remained in the intestine, particularly since it is well known that pituitrin has a stimulating effect on the intestinal musculature. Moreover, Rees(15) has tested this point and comes to the conclusion that while pituitrin does hinder the absorption of water from the intestine to a slight extent, this effect is not sufficient to account for the prevention of diuresis. Again, I found the same changes in the blood after water drinking whether pituitrin was injected or not.

Dale(16) and Motzfeldt(17) have found evidence of slight vasoconstriction of the kidney. King and Stoland(18), however, find that injection of pituitrin is followed by vasodilation in the kidney and diuresis. My observations so far as they went did not seem to indicate any great effect on the circulation.

Addis(19) denies that the inhibitory effect of pituitrin on the kidney is due to circulatory changes and attributes it to a specific action on the secreting cells of the kidney. Oehme(20) states that pituitrin inhibits the sensitiveness of the kidney to hydræmic stimulus. He finds it has no effect on the excretion of chloride. Von der Velden(21) finds that pituitrin causes retention of water and chlorides but not of phosphates and total nitrogen. Knowlton(22) denies that there is any evidence that pituitrin stimulates the kidney cells. On the whole the evidence seems to

be insufficient to decide the nature of the inhibitory action of pituitrin, but perhaps tends to show that the action is of a specific inhibitory nature on the excretory powers of the kidney cells. It is in agreement with this view that in my experiments the excretion of water and chlorides appears to be affected differently. Thus, during the time when the inhibitory effect of pituitrin is manifested the excretion of chloride falls much as it does during a day of starvation without pituitrin. When diuresis sets in however the excretion of chloride also increases steadily and tends to run parallel with the excretion of water. Without pituitrin on the other hand the excretion of chlorides, after a short initial increase, continues to fall during the time when the water diuresis is at its height. Also the excretion of phenol red seems to be unaffected by pituitrin in contrast to the great delay in excretion of water caused by this substance. Apparently the effect of pituitrin is to make the kidney cells less sensitive to diffusion pressure of water in the blood (cf. Oehme(20)).

Summary.

1. The diuresis consequent on water drinking is accompanied by changes in the blood (*a*) slight dilution (probably), (*b*) passage of salts (and possibly proteins) out of the blood.

2. The excretion of water is largely but not entirely independent of the excretion of chlorides.

3. Injection of pituitrin delays the diuresis produced by water drinking for 4–6 hours. Water diuresis then sets in and is accompanied by an increased excretion of chlorides.

4. It is suggested that the results recorded are compatible with regulation of excretion of water by the kidneys of a two-fold nature, (*a*) a main regulation dependent on the diffusion pressure of water in the blood, and (*b*) a subsidiary modification of this regulation dependent on the inability of the kidney to hold back water when the diffusion pressure of water in the urine is considerably below that of the blood.

In conclusion I wish to express my thanks to Dr J. S. Haldane for much helpful advice and criticism. The present paper is a direct continuation of work begun in conjunction with him in 1914.

I also wish to express my thanks to Dr G. Graham for help in some of the experiments and for suggestions with regard to the pituitrin observations and to Mr P. C. Raiment for assistance with some of the analyses of urine.

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ON THE RELATION OF PULSE PRESSURE TO THE OUTPUT OF THE HEART.

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THE pulse pressure is the difference between the maximum and minimum pressure in the arterial pulse curve—what are generally spoken of as the systolic and diastolic pressures. Since the rise of pressure in the arteries at systole is due to the propulsion of a certain quantity of blood from the left ventricle into the aorta, the pulse pressure will tend to vary with the amount of blood put out at each beat of the ventricle. It has been suggested therefore, that the pulse pressure might be taken as a measure of output; and that the pulse pressure multiplied by the pulse rate, when multiplied by some constant factor which might vary from individual to individual, might be equal to the output per minute or the minute-volume of the heart. On this hypothesis a number of estimations of pulse rate and pulse pressure were carried out by Erlanger and Hooker(1) on two men, and certain deductions were drawn therefrom as to the changes in the output of the heart and the velocity of the circulation. Since a definite relation between the figures so obtained and the minute volume is very generally accepted at the present time by clinicians, it was suggested to me by Professor Starling that it would be worth while to investigate the subject experimentally.

There are many difficulties in assuming a numerical proportion between the pulse pressure and the ventricular output. In the first place the "output time" is not confined to the rise of the pulse curve, but extends from the beginning of the rise to the dicrotic notch, and at this latter point the pressure may have sunk nearly as low as the "diastolic pressure." Only with a very high pressure and an anacrotic pulse will the rise of the pulse curve coincide in point of time and extent with the output of blood into the aorta. Moreover, the course of the pressure curve in the arteries is complicated by secondary waves, partly centrifugal, partly reflected and centripetal, which may diminish or increase its maximum, apart from alteration in the amount of blood entering the aorta from the heart. And it is clear that a short sudden contraction of the heart will raise the pressure more, and give a bigger pulse pressure,

than a slow prolonged contraction which expels the same amount of fluid; *i.e.* with a constant output per beat the pulse pressure will tend to be higher with a low arterial resistance than when the arterial resistance is high.

The extent of these disturbing factors can be determined only by experiment. If, for instance, it were found that the expression pulse pressure × pulse rate × a constant, only varied within, say, 50 p.c. from the actual output per minute of the ventricles, the method would still be of some value, in view of the very wide alterations in output that have been found to accompany changes in bodily conditions.

The question has been investigated on the heart lung preparation (2). In this preparation the output of the left ventricle can be readily determined; and it is possible to modify to any extent, and independently, either the amount of blood flowing into the heart, and therewith the

Exp. 1.

	Temp.	Blood pressure Max.	Blood pressure Min.	Pulse pressure	Heart rate per 10 secs.	P. P. × H. R.	Outflow c.c. per 10 secs.	P. P. × H. R. / outflow	Circulatory conditions
1	35° C.	88·0	66·0	22·0	22	484	21·6	22	Art. resistance moderate. Inflow beginning small, increased for 2, 3, and 4.
2	„	86·1	58·3	27·7	22	610	53·1	11·4	
3	35·5°	96·3	66·0	30·3	23	697	75·7	9	
4	„	175·0	86·1	88·9	24	2034	312·5	6·5	
5	„	235·5	150·4	85·1	24	2042	83·3	22	Art. resistance high. Inflow increased for 6.
6	„	256·8	147·3	109·5	24·5	2683	147·0	18	
7	30·0	153·5	100·0	53·5	15·5	829	36·0	23	Art. resistance fairly high. Inflow increased for 8 and 9.
8	29·5	163·0	88·0	75·0	14·5	1087	113·4	9·5	
9	30·0	188·2	88·0	100·2	15·0	1530	192·3	8	

Exp. 2.

A	35° C.	120·7	90·0	30·7	20·0	614	18·2	33	Art. resistance 60 mm. Inflow increased for B and C.
B	„	130·0	80·0	50·0	21·0	1050	64·0	16	
C	„	200·0	70·0	130·0	13·7	1781	147·0	12	
D	34·5°	220·0	150·0	70·0	20·8	1456	33·3	43	Art. resistance 135 mm. Inflow large.
E	34°	100·0	0·0	100·0	21·0	2100	200·0	10	Art. resistance zero. Inflow large, increased for F.
F	„	94·5	10·5	84·0	21·0	1764	227·0	7	
									Pericardium open:
G	„	120·0	80·0	40·0	21·0	840	58·0	14	Art. resistance 60 mm. Inflow increased for H.
H	„	170·0	75·0	95·0	23·0	2185	277·0	8	
K	„	230·0	135·0	95·0	26·0	2470	52·0	47	Art. resistance 132 mm. Inflow moderate.
L	„	73·5	5·0	68·0	22·0	1496	89·3	16	Art. resistance zero. Inflow moderate, increased for M.
M	„	115·0	20·0	95·0	24·0	2280	333·3	7	

output of the heart, or the resistance which the blood has to encounter in leaving the arterial system, and therewith the arterial pressure and the resistance to be overcome by the heart in expelling its contents. Moreover, by altering the temperature of the blood flowing into the heart, the frequency of the heart beat can be changed within wide limits.

Two experiments were made on preparations from dogs weighing from 8 to 9 kilos. Hirudin was not used, the preparation being kept going on defibrinated blood alone. In the first experiment the pulse pressure was determined by recording the oscillations of the arterial pressure close to the aorta by means of a Frank manometer, as made by Jaquet. This was connected by a rigid lead tube with the arterial system. The pericardium was retained intact throughout. In the second experiment the arterial pressure was measured by a Hürthle spring manometer. Observations were made with both closed and open pericardium. In each case the manometers were calibrated against a mercurial manometer at the end of the experiment.

It is quite clear from these results that the product of pulse pressure by pulse rate is of no value as a measure of heart output. In the experiments the relation between these two quantities varied between 6·5 to 1 and 47 to 1. A method of calculating output which can give such widely varying results is obviously of no use in clinical practice, and any attempt to draw conclusions as to output from a determination of pulse pressure and pulse rate can only lead to error.

I am indebted to Professor Starling for performing the operations in preparation for these experiments.

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EXOSMOSIS FROM ANIMAL CELLS.

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In a previous publication (1) it has been shown that when living trout eggs are injured there is a very marked evolution of electrolytes to any medium whose concentration of intracellular electrolytes is less than that of the interior of the egg. It was also shown that the mechanism whereby healthy eggs retain their electrolytes must lie in the protoplasmic membrane. The following experiments were performed to investigate more fully the method by which exosmosis occurs in toxic solutions.

The exosmosis from injured cells is, of course, a well-known fact, but detailed knowledge of the phenomenon is limited. The only satisfactory means of measuring the rate at which electrolytes leave a cell is to estimate (by electrical or other means) their concentration in the surrounding medium at various stages of the reaction. Such experiments have been performed in the case of vegetable cells by Jørgensen and Stiles (2), while Stewart's (3) results lead to the conclusion that similar processes are at work in the case of red blood-corpuscles. In all cases the experiments have been performed with complete tissues, or with a large number of cells.

It is, however, well established that the individual cells composing a tissue (or the individual members of a unicellular population) show wide variation in their resistance to such toxic reagents as are used to induce exosmosis (Peters (4), Brooks (5)). This being so the rate of loss of electrolytes from a bulk of cells is no indication of the rate of loss from any individual cell. It is simply to show that the eggs of a single rainbow trout represent a highly heterogeneous population when estimated by their resistance to such agents as alcohol or aniline. The eggs are peculiarly satisfactory material for such investigations, since the precipitation of the intracellular globulins provides a simple index for the process of exosmosis (see Gray (1)).

Table I shows the percentage of eggs in which exosmosis took place when treated with ethyl alcohol in varying concentrations.

It is clear that except when treated with ethyl alcohol of high concentration, the eggs show a wide range of resistance to the reagent. It

TABLE I.

Time in hours	70 p.c.	35 p.c.	21 p.c.	14 p.c.	7 p.c.	0 p.c.
1½	100	0	0	0	0	0
4	—	15	0	0	0	0
22	—	100	40	20	8	0
27	—	—	48	32	8	0
45	—	—	100	35	10	0
70	—	—	—	85	15	0
120	—	—	—	90	18	0

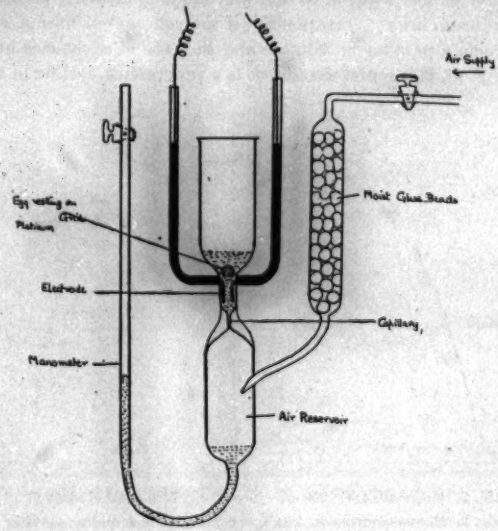

Fig. 1.

follows that the rate at which the electrolytes are given off from a considerable number of cells is no index of the rate of exosmosis from a single cell, unless the toxicity of the solution used is so great that all cells are equally affected within a very short time. This is the case in 70 p.c. ethyl alcohol. Fig. 2 shows the rate at which electrolytes diffused out of 200 eggs into 30 c.c. of 70 p.c. alcohol.

It is obvious that the rate of exosmosis from a cell can only be studied by the use of single cells. This was found to be possible by means of the apparatus here figured (Fig. 1), which was kindly lent to me by Prof. V. H. Blackman. Exosmosis was measured by determinations

of the electrical conductivity of the external medium. Fig. 3 shows the rate of exosmosis from a single cell into 0·7 c.c. of 0·2 Mol. aniline solution. It will be seen that the general shape of the curve is identical with that shown in Fig. 2. At first the rate of exosmosis rises to a maximum, it then begins to fall off. The first part of the curve represents the period during which the cell membrane is breaking down. Once the whole membrane has been destroyed over the entire surface of the egg, the electrolytes diffuse away into the surrounding medium according to simple diffusion laws. Consequently, if the contents of normal eggs are placed inside a parchment thimble and the rate of exosmosis be determined (Fig. 4), it is found that there is a progressive decline in the rate

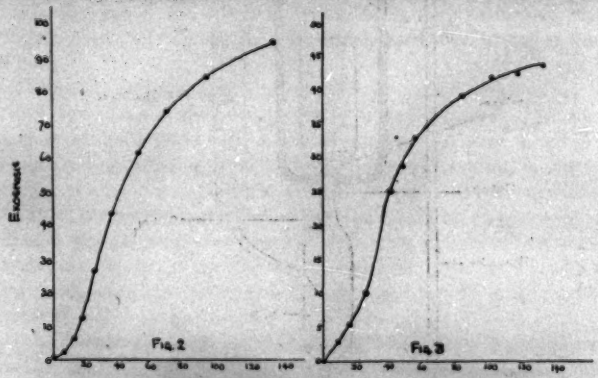

Fig. 2. Exosmosis from 200 eggs into 30 c.c. 70 p.c. ethyl alcohol.
Fig. 3. Exosmosis from one egg into 0·7 c.c. 0·2 Mol. aniline solution.

of exosmosis, identical in its nature with that illustrated during the latter stages of normal exosmosis.

Bearing in mind the wide degree of variation between different eggs of the same female fish, it is interesting to consider the action of different strengths of toxic reagents on the rate of exosmosis. A large number of experiments were performed, of which those shown graphically in Fig. 5 are examples. The results show quite clearly that the absolute rate of exosmosis from different eggs in the same solution are simply an index of the susceptibility of the different cells. They also show that the general nature of exosmosis is the same in all cases. There is an initial phase during which the cell membrane is destroyed; the length of this

phase depends upon (*a*) the resistance of the individual cell, (*b*) the strength of the toxic solution. Once this initial phase is over, the electrolytes diffuse out of the cell according to simple diffusion laws. There is no evidence to support the view that the intracellular electrolytes represent the equilibrium of such a system as is postulated by Donnan(6) or by Moore(7).

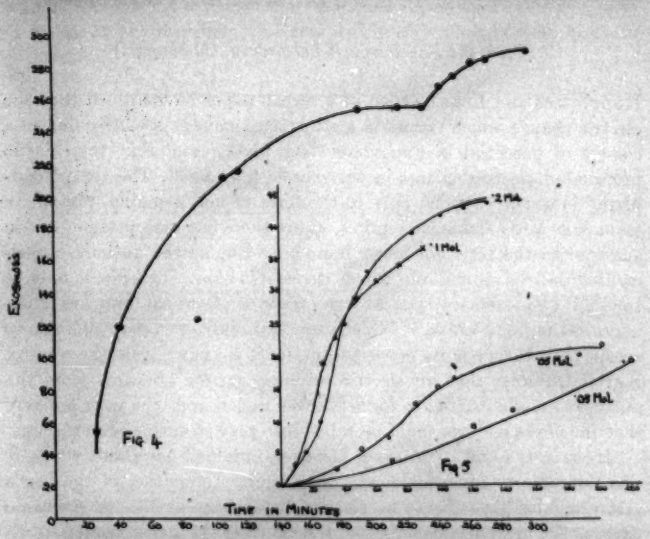

Fig. 4. Diffusion of electrolytes through a parchment membrane from 5 c.c. egg contents into 30 c.c. distilled water. At 3 hours 50 minutes (230 minutes) after the beginning of the experiment the water outside the membrane was changed.

Fig. 5. Rate of exosmosis of electrolytes from single eggs into 0·7 c.c. aniline solutions.

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THE ELECTRIC RESPONSE OF DENERVATED MUSCLE.

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Henriques and Lindhard(1) in a recent paper have argued that the electric change which occurs in a stimulated muscle is wholly due to a change of potential in the motor "end-plate," and that there is no propagated electric change in the muscle fibre itself. The term "end-plate" is usually applied only to the form of nerve ending, present in mammals, birds and some reptiles, where there is a sarcoplasmic sole in addition to the terminal nerve branches. But as the authors experimented on frog's muscle in which the sole is absent or present only in traces, they presumably mean by end-plate the terminal branches of the nerves—the "end-brush." Whichever they mean it is very difficult to accept their theory in its present form, for it is known from the work of Keith Lucas(2) that an electric response can be obtained from the sartorius muscle excited in its nerve free region and it is very unlikely that the nerve endings could be called into play in such circumstances.

Henriques and Lindhard, however, obtained one result which it was desirable to investigate, viz. that direct stimulation of the frog's gastrocnemius gave rise to no electric disturbance at all. At the same time, as the most direct method of testing their theory, it seemed worth while to determine whether any change in the electric response of a muscle occurs when the nerve endings have degenerated.

In a number of frogs anæsthetised with ether, the sciatic nerve was cut high up in the thigh so that the nerve both to the sartorius and the gastrocnemius was severed. At different times after the operation the frogs were pithed, one or both muscles of either side were cut out and the electric changes in them compared. Most of the observations were made on the sartorius, as the simple arrangement of the muscle fibres in it makes the analysis of electrical changes much easier than in the more complicated gastrocnemius. The sartorius was stimulated at the pelvic end before and after destroying the tibial end with a hot wire, and the electric response was led off by electrodes on the tibial end and 16–20 mm.

away. The electric responses were photographed by means of the capillary electrometer and the records corrected by Lucas's machine. Duplicate records were taken and calibration curves were made before and after damage. After each experiment the sartorius muscles were treated with methylene blue; 19 days after the operation the nerve endings had disappeared from most of the muscle fibres on the denervated side and were represented only by a row of fine dots in the remainder. After 36 days no traces of nerve ending were found. The experiments were made in the summer.

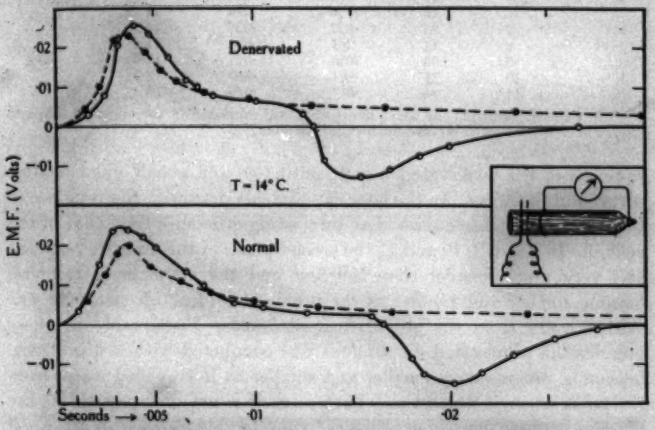

Fig. 1. Electric response of sartorius muscle, diphasic and monophasic. Below, normal muscle with 20 mm. between leading off electrodes. Above, muscle from opposite leg 19 days after section of nerve, 16 mm. between electrodes.

None of the denervated muscles failed to give an electric response. A typical record is shown in Fig. 1, which gives the analysed curves for the response of a sartorius 19 days after section of the nerve and below it the response of the control muscle from the other leg. The dotted lines record the monophasic response taken after damage to the tibial end. The rate of conduction is slightly faster in the denervated muscle than in the control, being 1·4 metres per sec. in the former and 1·2 in the latter, but in other respects there is very little difference between the two in spite of the absence of nerve endings in one muscle.

The maximum potential difference developed in the control and the

denervated muscles at different times after nerve section is shown in Table I, and it will be seen that there is no sign of any diminution in the

TABLE I.

Exp.	Days after nerve section	Maximum potential (volts) denervated muscle	Maximum potential (volts) control	Temperature C.
1*	3	·030	—	14
2*	6	·0225	—	13·5
3*	9	·038	—	15
4	12	·027	·029	14
5	15	·0325	·036	14·5
6	19	·026	·024	14
7	35	·037	·037	14
8	42	·041	·034	11
9	55	·025	·027	10·5
10	71	·021	·037	12·5
11	94	·011	·026	15

* In these experiments the nerve fibres were still excitable on the operated side and the nerve endings showed no obvious histological changes.

response of the denervated muscle until two and a half months after division of the nerve. In the later experiments, however, the response of the denervated muscle showed an interesting difference from that of the control. In Exps. 8, 10 and 11 the second phase of the diphasic response was very much smaller than the first and the monophasic response became smaller and smaller as the proximal leading off electrode was shifted away from the stimulating electrodes. Presumably in these muscles the propagated disturbance was conducted with a decrement, becoming progressively smaller and smaller as it travelled away from the point where it was set up. Such a result is not unexpected, for the muscles in these experiments showed considerable fibrosis; in a normal muscle it is known that the disturbance is conducted with a decrement after fatigue has set in, and in a nerve fibre almost any abnormal conditions may give rise to this type of conduction. The rate of conduction was not appreciably slowed except in Exp. 11, but in the last three experiments the monophasic response subsided more slowly in the denervated muscles.

In these experiments it is unlikely that the nerve endings would be called into play at all, since the stimuli were confined to the pelvic end of the muscle. It was still possible therefore that the nerve endings might contribute something to the total response when the stimulus takes effect on the nerve trunk, and to test this point two experiments were made in which the electric response of a sartorius to direct stimulation was compared with that to indirect stimulation via the nerve. The

nerve to the sartorius was dissected out and the muscle was set up as shown in Fig. 2 with stimulating electrodes applied either to the nerve or to the pelvic end of the muscle and leading off electrodes on the tibial end and midway between the pelvic end and the middle of the muscle. The strength of stimulus was adjusted to give a maximal contraction in each case, but it was found that the contraction in response to a stimulus at the pelvic end of the muscle was always slightly smaller than that given by stimulating the nerve trunk.

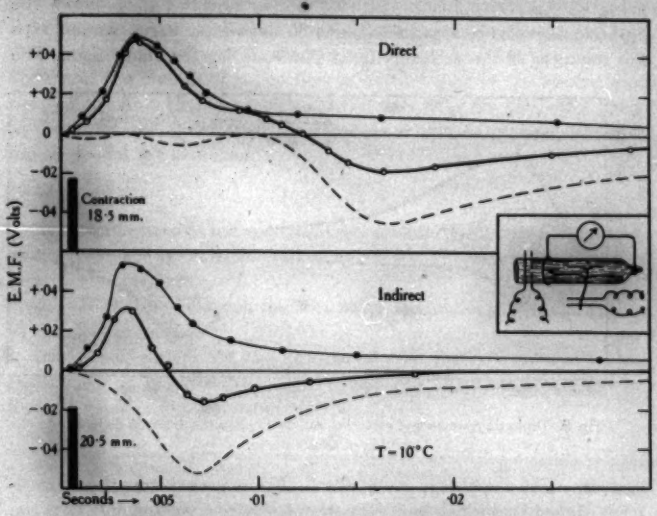

Fig. 2. Diphasic and monophasic responses to stimulation of muscle in pelvic region (above) and of motor nerve trunk (below).

The results of one of these experiments is shown in Fig. 2. If we consider first the monophasic responses we find the maximum potential difference on indirect stimulation to be ·053 volt and that to direct ·049 volt, *i.e.* the former is 8 p.c. larger than the latter. But the contraction with indirect stimulation is 10 p.c. larger than that with direct so that for equal contractions there should be little or no difference in the electric responses. The diphasic response to stimulation via the nerve gives a much smaller potential than that to direct stimulation of the muscle, but this is due to the early arrival of the electric change at the

tibial electrode and the resulting interference between the two phases of the response. This may be seen from the dotted lines in Fig. 2 which give the response at the tibial electrode as calculated by compounding the observed monophasic and diphasic curves. Thus the excitation of the nerve endings during the passage of a propagated disturbance from nerve to muscle can contribute next to nothing to the total electric response.

As we have said, an electric response was obtained from the denervated gastrocnemius as well as the sartorius. Fig. 3 shows the diphasic response of a gastrocnemius 55 days after nerve section with the response of the sartorius from the same leg for comparison. The

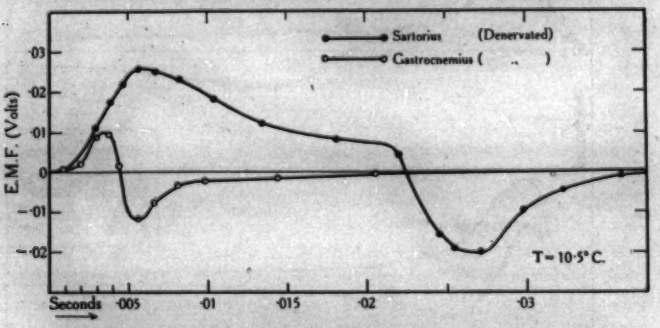

Fig. 3. Diphasic responses of sartorius and gastrocnemius, both 55 days after section of sciatic nerve.

gastrocnemius response is small, as would be expected from the arrangement of the fibres which limits the stimulation to a portion only of them. The short length of the fibres causes great interference between the two phases, but it is impossible to record a pure monophasic response as we cannot damage the end of every fibre without destroying the muscle.

In certain conditions we have been able to repeat Henriques and Lindhard's observation that a stimulus to the gastrocnemius may give a contraction without any electric response, but we have only found this when the stimulus is weak and the leading off electrodes are shifted as far as possible from the stimulating electrodes. In this case the stimulus only affects fibres at one end of the muscle and the tissue between the two leading off electrodes remains inactive. If the leading off electrodes

are arranged to include with certainty the fibres which are active we have never failed to detect an electric response.

In view of the facts given above it is unnecessary to consider the arguments of Henriques and Lindhard in detail, but one may be mentioned. As is well known, a diphasic response may be obtained from a muscle when one leading off electrode is on the muscle and the other on the tendon or bone or on some other indifferent part of the body. They consider that, on the accepted view, such an arrangement should give a monophasic response because the disturbance can only pass under the electrode which is on the muscle. This argument overlooks the fact that any inactive tissue intervening between the electrode and the surface of the muscle fibre becomes as much a part of the electrode as the clay or damp thread which makes contact with the tissues. As long as the fibres are undamaged the response should be diphasic however the electrodes are arranged.

Summary.

1. Stimulation of the denervated sartorius and gastrocnemius muscles of the frog sets up a diphasic action current as it does in muscle with intact nerve endings.

2. There is no evidence that the nerve endings contribute anything to the electric response.

3. In our experience the absence of the electric response in the gastrocnemius described by Henriques and Lindhard only occurs when the leads are taken from inactive muscle.

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(1) Henriques and Lindhard. Arch. f. d. ges. Physiol. 183. p. 1. 1920.
(2) Keith Lucas. This Journal, 39. p. 333. 1909.

THE IDENTITY OF HÆMOGLOBIN IN HUMAN BEINGS.

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(*From the Physiological Laboratory, Cambridge.*)

THE different affinities for oxygen of various samples of human blood may be due either (1) to differences in the hæmoglobin itself, or (2) to differences in the conditions affecting it, *e.g.* the salt and hydrogen ion concentration. Whilst it seemed unlikely that there are any considerable differences in the hæmoglobin of different persons, it was desirable to determine experimentally whether this was the case or not; and in this paper we give an account of our investigations.

In the first place, the dissociation curves of a number of persons were determined. The method of preparation which seemed most suitable was based on dialysis. The hæmoglobin of human blood crystallises with difficulty even in the presence of ether and our idea, at first, was that such reagents as alcohol and ether should be avoided. We commenced therefore by adopting the following technique.

About 3 c.c. of corpuscle suspension, washed twice with ·8 p.c. NaCl, were run into a sterile collodion membrane, leaving about $\frac{1}{2}$ c.c. air space. The membrane was closed with a screw clip faced with rubber. Laking of the corpuscles appeared to occur during the first hour of dialysis; and the process was hastened by running the air bubble up and down the tube a few times. The dialysis was carried out in a cylinder with 200 c.c. of distilled water kept at 0° C. and changed at least three times a day for two days. By this means a sample of hæmoglobin, about 2–3 c.c., was obtained containing at least as much hæmoglobin as the same amount of blood, and at least as pure as that of Robert's preparation. The sample was transparent or translucent. It was somewhat viscous and only filtered with difficulty. One sample set into a gel on cooling with ice. The dissociation curves are shown in Fig. 1. It will be seen that these curves differ widely.

The most obvious explanation of the cause of difference was that the hæmoglobin changed in its salt content during the course of the experi-

ment. It need hardly be said that in dealing with solution of high resistance, equivalent in one case to that of 0·6 mg. of NaCl per litre, the utmost care is necessary in the cleaning of all the glass, etc. with which the hæmoglobin solution comes into contact. A control experiment in which water was used instead of hæmoglobin showed that the conductivity of the water was practically the same at the end of the experiment as at its commencement. Casual contamination with electrolytes therefore could not be held to explain our results.

There is, however, a more subtle possibility which demands some

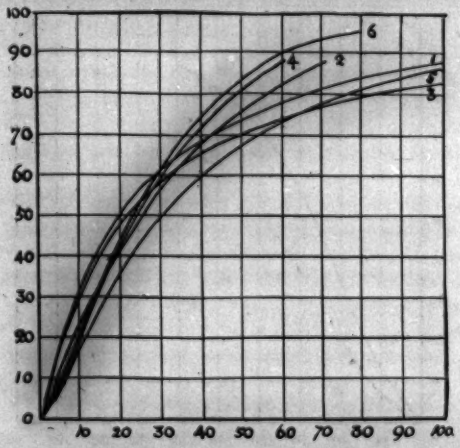

Fig. 1. Ordinate = percentage saturation of hæmoglobin with oxygen. Abscissa = oxygen pressure in mm. Curves for preparations made by dialysing corpuscles against distilled water: 1 Parsons, 2 Mrs Parsons, 3 Collis, 4 Priestman, 5 Shoji (2), 6 Shoji (1). Similar curves for Barcroft and Bock are shown in Fig. 3.

consideration, as was pointed out to us by A. V. Hill, namely, that the hæmoglobin may only yield up the last traces of electrolyte saline slowly. So that from intrinsic causes the conductivity of the solution might rise during the course of the experiment. He therefore suggested that instead of dialysing our solution against distilled water alone we should subsequently use a standard salt solution. This we did, but in the event we found scarcely a greater degree of uniformity than we did in our distilled water solutions. The curves of each subject's hæmoglobin need not be considered, but two cases will suffice, they are shown in Fig. 2.

It is clear that no greater degree of uniformity is to be obtained from dialysis against salt solution than against water. Are we then to conclude that the hæmoglobin of different individuals differ? Against doing so, there is one consideration which requires mention, namely, that specimens of hæmoglobin from the same person taken at different times and dialysed against distilled water differ as much from one another as do specimens from different persons.

An example may be seen in Fig. 1. Two curves labelled Shoji (1) and Shoji (2) were obtained from samples of blood taken within a week.

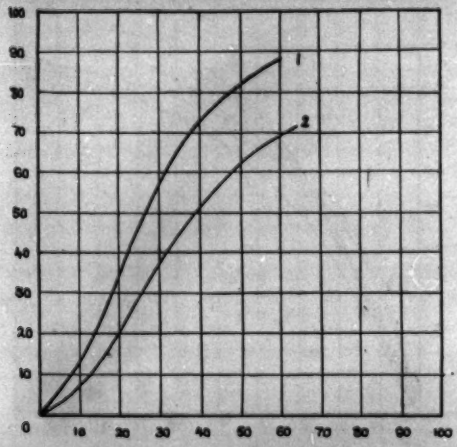

Fig. 2. Ordinate and abscissa as in Fig. 1. Oxygen dissociation curves of two samples of hæmoglobin from blood dialysed first against distilled water and subsequently against 0·9 p.c. NaCl solution: (1) from Parsons, (2) from Mrs Porter.

They differed greatly; that labelled Shoji (2) being rather similar to the Barcroft-Roberts[1] curve for dogs, that labelled Shoji (1) being much more evidently S-shaped. The principal difference between the solutions appeared to lie in their conductivity. The table on p. 335 gives the conductivities of the hæmoglobin solutions, the dissociation curves of which are given in Fig. 1.

A possible hypothesis was that the solutions with the lowest conductivity agreed most closely with the Barcroft-Roberts curve and that as the saline concentration increased the curve became more infected.

[1] This *Journal*, 39. p. 143. 1909.

Subjects	Conductivity in equivalents of NaCl
Collis	6 mg. per litre
Mrs Parsons ...	3·3 ,,
Shoji (2)	50 ,,
Bock	131 ,,
Shoji (1)	187 ,,
Priestman ...	220 ,,

The general body of evidence did not, however, bear out this theory, and moreover, the Barcroft-Roberts solution of dog's hæmoglobin had a conductivity corresponding to 144 mg. NaCl per litre.

Having failed so far to account for the irregular character of our solutions we tested the effect of absolute concentration of the hæmoglobin. For this purpose the strongest possible solutions were made and divided into two portions, one of which was diluted to half its concentration preferably by dilution with the water against which it had been dialysed. The two then had their dissociation curves determined. In general that of the more dilute solution fell somewhat above that of the more concentrated one, not crossing it at any point. It is of course very difficult to dilute a solution of hæmoglobin without altering its properties in other respects. If the dialysing water be used for the purpose, it is probable that the conductivity of the solution will be decreased as the hæmoglobin has a certain—or uncertain—conductivity of its own. If, on the other hand, the conductivity of the water used for dilution be artificially brought up to that of the hæmoglobin solution there is a great chance of changing the hydrogen ion concentration.

We were, however, unable to satisfy ourselves that variability of the concentration of our hæmoglobin solutions accounted completely for the divergence of our results.

Whilst these experiments had been taking place, another property of the solutions had gradually been forcing itself upon us, namely, their transparency. Some of our solutions were more transparent than others. We therefore subjected them to microscopical examination. We had supposed that in the hæmoglobin solution there might be "shadows" which owing to the viscosity and the high specific gravity of the solution, it had been impossible to separate by centrifuging. One film which we made rather bore out this view for when stained with Jenner's stain the solid matter did not take the stain whilst the solution stained red. The nature of the material with which we were working revealed itself in the following way. If a drop of a relatively transparent solution of hæmoglobin be placed on a slide and a coverslip be put on it (thus pro-

ducing a thin layer of the material) the material presents a very ill-defined appearance. Clearly there is something solid in it, but it is very difficult to say what, or to describe it. If, however, a drop of saline is placed at the side and allowed to diffuse under the coverslip a remarkable transformation takes place. At once the preparation assumes the appearance of blood. The corpuscles are somewhat ill-shapen as is the case when a solution not quite isotonic with it is added to blood, but it is clear that they retain a great part of their hæmoglobin. If instead of a microscopic preparation, a little of the original dialysed "rather transparent" solution be taken in a test tube and some salt added, it immediately assumes the appearance of normal blood.

Clearly the solutions with which we had been working were not solutions in the accepted sense at all. They consisted of a mass of corpuscles which as dialysis had proceeded gradually swelled up at the expense of the surrounding fluid till they finally almost lost their refrangibility. Consequently it became possible to see light through the solution. It now seemed that a possible explanation of the irregular behaviour of our solutions was forthcoming. The dissociation curve of hæmoglobin even within the saltless corpuscle might be essentially different from that of free hæmoglobin in a correspondingly salt-free solution. It remained to prepare solutions of hæmoglobin, making quite certain that the corpuscular elements were all absent and to test the identity of the curves which these solutions yielded. Two persons were chosen, Bock and Barcroft, whose hæmoglobin when dialysed against water had yielded very different curves (cf. Fig. 3). From the blood of these, samples of hæmoglobin were made as follows.

About 50 c.c. of blood were drawn from each subject. After defibrination and centrifuging with 8 p.c. NaCl, the cake of corpuscles in each tube was laked by the addition of ether, about 50 p.c. by volume of the corpuscles. The mixture was well shaken, and the tubes immediately placed on ice and left for an hour. After centrifuging for a short time, the preparations showed three well marked layers: (1) ether at the top, (2) a pale pink layer of stroma, and (3) at the bottom a deep red clear hæmoglobin solution. The ether and pink gelatinous stroma were partly sucked off and then a fine pipette was pushed down and the hæmoglobin solution sucked out—a portion of the ether was removed by bubbling air through, and the solutions of hæmoglobin were placed in two collodion membranes and clipped. After 2½ days' dialysis, the conductivity of the two solutions was equivalent to a ·00485 solution of NaCl taking the $N/10$ as the standard for comparison.

The dissociation curves yielded by these solutions appear to be identical and to bear no evident resemblance to the curves of either Bock's or Barcroft's hæmoglobin when in, or largely in, their dialysed corpuscles.

On looking back through our results we have found one dissociation curve which appears to be identical with that obtained from Barcroft's and Bock's blood by freeing hæmoglobin with ether. This curve was obtained from a sample of Redfield's hæmoglobin prepared by treatment

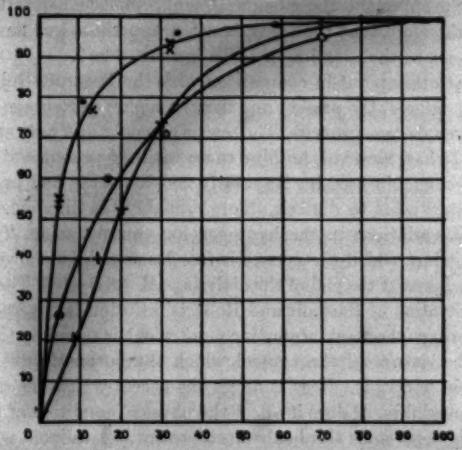

Fig. 3. Ordinate = percentage saturation of hæmoglobin with oxygen. Abscissa = oxygen pressure in mm.

● Barcroft; hæmoglobin free from corpuscles. × Bock; ditto.
○ Barcroft; blood dialysed against distilled water. + Bock; ditto.

with aluminium hydroxide and was free from corpuscular elements. At the time the difference between the aluminium preparation and all the others was so great that we were inclined to think the alumina had some specific action. This solution had a conductivity = 80 mg. NaCl per litre. The points determined were as follows:

Oxygen pressure ...	3	8	11·5	22	38
Percentage saturation	22	66	76	87	92

The above observations appear to have raised more points for discussion than they have settled.

(1) What is the precise shape of the curve which represents salt-free

hæmoglobin? The first phase of this question is whether or not the curve is a rectangular hyperbola. Placed as it is, so near both the ordinate and the abscissa over a great part of its course the answer is difficult to give. Like the Barcroft-Roberts curve a hyperbola might be drawn at such a distance from it that all the observed points would approximate, within the range of experimental error, to the hyperbola. Such a curve would in this case have an equation of about $xy = 400$. Nevertheless all the points at oxygen pressures of above 15 mm. would be above the curve and those below 10 mm. would be below it. It seems probable therefore that the curve is not a hyperbola but has a definite inflection, *i.e.* in the notation of Hill's formula $n > 1$.

(2) The other point in comparison with the Barcroft-Roberts curve is evident, namely, the present one shows a higher percentage saturation for any given oxygen pressure. The reason can only be a matter for future research. It has, however, nothing to do with the presence of corpuscles for the hæmoglobin used by Barcroft and Roberts had been crystallised by ether prior to dialysis. More probably the difference has to do with slight variations in the hydrogen ion concentration. That of the solution against which the corpuscle-free hæmoglobins shown in Fig. 2 was dialysed was at the end of the dialysis $p.H = 8{\cdot}0 \pm 0{\cdot}5$. The hydrogen ion concentration of Barcroft and Roberts' solution is not known.

(3) Perhaps the most interesting point which is raised by the observations is the nature of the control which the corpuscle exerts on the hæmoglobin within it. Does it affect the access which the oxygen has to the hæmoglobin, or does it affect the physical condition of the hæmoglobin or is there some third substance present, *e.g.* a lipoid which forms an integral part of the oxygen-hæmoglobin complex?

Conclusion.

The observations made seem to show that if samples of hæmoglobin from different individuals be freed from the corpuscles in which they were held and be subsequently treated in exactly the same way they give oxygen-dissociation curves which are indistinguishable. On the other hand, preparations prepared by slow dialysis of blood against distilled water may simulate solutions in appearance but still containing intact, though swollen corpuscles give dissociation curves far different from those of true aqueous solutions of hæmoglobin.

The expenses of the above research have been borne largely by the Medical Research Council, in part by a grant from the Proctor Fund, Harvard University.

THE DIRECT MEASUREMENT OF THE PARTIAL PRESSURE OF OXYGEN IN HUMAN BLOOD.

By J. BARCROFT and M. NAGAHASHI.

(*From the Physiological Laboratory, Cambridge.*)

A direct method for the measurement of the oxygen pressure in the arterial and venous blood of man is much to be desired. The following procedure in which there is little that is new, but which is rather a combination of ideas introduced at various times by a number of workers, Krogh(1), Barcroft and Hamill(2), Brodie and Cullis(3) and Stadie(4), has given us results which seem satisfactory.

In principle, the method is as follows. Blood is withdrawn from a vessel, artery or vein by direct puncture; to this blood is exposed a small bubble of alveolar air at 37° C. until an equilibrium is established between the blood and the bubble. The bubble is then analysed in a suitable apparatus.

In detail the procedure is carried out as follows:—the blood to the volume of 10 c.c. or rather more is withdrawn by means of a syringe the needle of which must fit perfectly so that no air is sucked in at the junction. In our first experiments the air bubble was introduced into this syringe. We gave up this procedure for two reasons: (1) the syringe being lubricated with paraffin, a film of the oil separates the bubble from the blood and equilibrium takes place with difficulty. (2) The end of the syringe being flat inside, the bubble cannot be expelled with certainty when the time comes for its transference to the analyser. When the blood is withdrawn therefore it is at once transferred to an all glass syringe with a barrel sufficiently well ground to make it air-tight in use, and with a plunger conical at the end. As supplied by the maker the syringe terminates in a cone and ends abruptly at the level of the end of the plunger, there being no nozzle. On to this end was blown a small tap with 2 mm. bore tubing. The whole is represented in the figure.

This syringe is prepared for use in the following way. The plunger is taken out, the tap is shut, a few grains of hirudin are put into it, but not suffered to adhere to the sides. The plunger is moistened with saliva

and replaced. A little mercury is drawn into it. A nose-clip is put on the nose, the nozzle of the syringe with the tap open is put between the teeth, the lips are almost closed and a forced expiration is made, at the end of which the lips are completely closed and alveolar air from the mouth is drawn into the syringe and most of it expelled again. The lips are now opened and the syringe removed. The tap is opened ever so little and all the alveolar air in the syringe with the exception of $\frac{1}{10}$ c.c. is expelled. The syringe is now inverted so that the nozzle points downwards. The bubble rises, or if necessary is shaken above the mercury, the tap is opened and the dead space is filled with mercury from within the syringe.

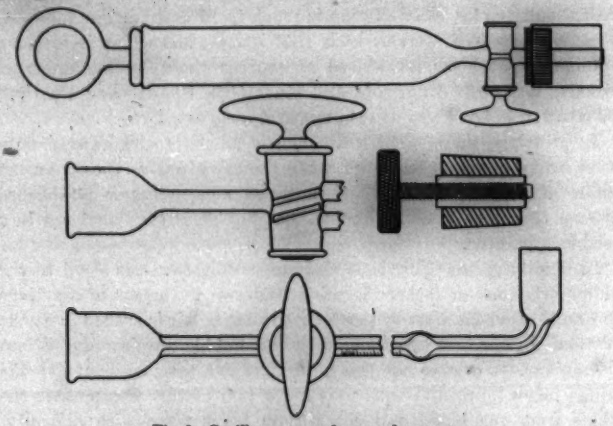

Fig. 1. Capillary gas analyser and saturator.

A small piece of 1 mm. bore rubber tubing is placed on the nozzle, the lumen of this is also filled with mercury. To this syringe the blood is transferred without exposure to air. It is rotated for ten minutes in a water bath at 37° C. and then the bubble is transferred to the gas analyser.

The method of transfer will be discussed later.

The analyser consists of a piece of glass tubing of 1 mm. bore at one end, the tubing is turned at right angles and broadens out into a small cup; at the other end is a three-way tap as shown in the figure. About a centimetre from the cup the lumen is expanded into a spherical chamber of about $\frac{2}{10}$—$\frac{3}{10}$ of a cubic centimetre capacity. The portion of tubing

between this chamber and the tap is graduated in centimetres and millimetres on the glass. This portion should be about 17 cm. in length. Beyond the three-way tap is a cup of about 1·5 cm. diameter. Into this cup goes a well fitting rubber cork and through the cork a steel cylinder containing a screw plunger. Perhaps our greatest difficulty has been to obtain a sufficiently air-tight screw.

The analyser is prepared as follows:—the tap is shut, the cup filled to about one-half its capacity with mercury, the cork greased and put into its place (which of course causes some compression of the air in the cup). The apparatus is then inverted so that the thumb-screw is downward, the tap is then opened and the cork driven home. The air of course escapes and there should be enough mercury to fill the tube as well as the cup. The cork is then tied in its place by a piece of tape which passes round the tap and under the cork. If there is any air left in the cup or if there is too much mercury in it the undesired material may be expelled through the orifice of the tap which leads to the air. The apparatus now being full of mercury, a little water is put in the small cup, drawn into the tube by turning the thumb-screw and expelled. The object is to make a film of moisture on the inside of the graduated part of the tube. The analyser is now ready to receive the bubble.

In our first experiments the transfer was made over mercury by means of a convenient attachment which need not be described as the method was discarded subsequently. The bubble then being in the small inverted cup, is withdrawn into the tubing. The analyser is now removed. The small cup (which for the sake of distinguishing it from the large mercury cup, we shall call the gas cup) is placed with its orifice upwards, a few drops of special saline are put into it. The saline is drawn in. By dint of a little manipulation, the air bubble is withdrawn entirely into the graduated portion of the tube, whilst those portions of the apparatus between the bubble and the gas cup are filled with saline. The presence of mercury in this chamber is immaterial so long as it is not present in sufficient quantity to close the tubing and so exert a pressure on the bubble. The gas cup should be emptied of excess of fluid. The saline used is prepared as follows: a few drops of octyl alcohol are placed in Ringer's solution. As much of the alcohol as will is dissolved, the remaining droplets are removed with a separating funnel. This fluid is placed in a large flask fitted with a rubber cork, pierced by a piece of glass tubing on which is a tap. Into the air of the flask is forced a volume of carbonic acid equal to about 6 p.c. of that of the flask. The flask is then inverted and the saline can be obtained through the tap.

The length of the bubble is now measured. For this purpose the analyser is placed horizontally up to the tap in an unsilvered cylindrical vacuum flask. It is thus protected from the immediate incidence on it of radiant heat, and in an ordinary room of equable temperature the bubble in ten minutes or so settles down to a uniform length. Under the analyser and also in the vacuum flask is a slip of mirror in order to assist the observer to avoid parallax.

When the initial length of the bubble has been measured, it is expelled into the chamber, into which 10 p.c. KOH is then drawn. The bubble by the same process having been drawn back into the graduated tube. If this is done with the tube as nearly vertical as possible, the fluids in the chamber will appear to be in layers, the saline being above the potash.

By altering the position of the analyser so that the gas cup is at a higher level than the tap and then making the bubble pass into the chamber once more the layer of saline may be driven out and more KOH sucked in. In a few minutes the CO_2 will all have been absorbed from the bubble, water is made to replace the KOH, the tube as well as the chamber being washed and the bubble now free from carbonic acid is measured.

We have never been able to get what we regard as accurate readings for the CO_2. The apparent readings have always been too small. This we take to mean that CO_2 has been lost from the bubble in the initial manipulation when it was in contact with saline. The initial length of the bubble is therefore too short and a correction must be made for this loss with which we shall deal later. The absorption of oxygen with pyrogallic acid is carried out in the same way as that of CO_2 with KOH.

If A be the length of the bubble before pyrogallic acid, B that after the acid, C the corrected initial length of the bubble, P the height of the barometer and T the tension of aqueous vapour in the bubble as it existed in the syringe (47 mm. for 37° C.) and O the pressure of oxygen in the blood in the syringe

$$O = \frac{100\,(A-B)}{C} \times P - T.$$

In the case of arterial blood the initial length of the bubble is calculated on the assumption that the pressure of CO_2 in blood was that of the alveolar air. In venous blood we have assumed that it was 50 mm. unless the blood was either very red or very black, in which case, a special calculation on the basis of the respiratory quotient and the CO_2 dissociation curve may be made.

The first tests to which we put the above method were carried out at room temperature. About 12 c.c. of blood was put in a saturator with a mixture of oxygen, nitrogen, and carbonic acid. The blood and the gas were thoroughly shaken until equilibrium between the two was established. The blood was then withdrawn into the syringe and its oxygen pressure estimated. The gas in the saturator was also analysed. It is clear that the oxygen pressure in both cases should be the same. The following consecutive comparisons were obtained:

Pressure of oxygen in bubble mm.	Pressure of oxygen in tonometer mm.	Initial pressure of oxygen in bubble mm.
38	38	35
21	19	110
19	19	110
17·5	16·5	16·5

The above seemed to indicate, as was to be expected from Krogh's work, that the method was worth following up. We therefore endeavoured to repeat the tests, exposing the blood in the saturator to a temperature of 37° C. In these circumstances we found that the oxygen tension as measured by the bubble was below that in the saturator.

	Pressure of gas in bubble mm.	Pressure of gas in saturator mm.	Initial pressure of gas in bubble mm.
(1)	25	29	28
(2)	37	41	110 approx.
(3)	35	41	110 „

We attributed the loss of oxygen to the cooling of the blood to which the bubble was exposed during its transfer from the syringe to the analyser. The hæmoglobin at the lower temperature would tend to absorb oxygen from a gas with which it had been in equilibrium at 37° C.

We therefore so modified the method that the bubble was transferred at 37° C. The saline bath used for the rotation of the syringe was made of a glass jar large enough to submerge the syringe either in the horizontal or vertical position. A short piece of glass tubing, larger in diameter than the gas cup, was fitted with a cork so that it could be attached to the nozzle of the syringe. With it the bubble may be transferred as follows: the gas cup is filled with saline and inverted over the edge of the bath which for the present purpose became a pneumatic trough. The syringe was placed vertically with its nozzle inside the gas cup and the glass tubing outside, the tap on the syringe is then opened and the contents gradually expelled. The bubble of course comes out immediately

and is at once drawn into the analyser whilst a gradual stream of blood is kept running through the gas cup by pressure on the handle of the syringe. The following determinations were made in this way:

Pressure of oxygen in saturator mm.	Pressure of oxygen in bubble mm.	Initial pressure of oxygen in saturator mm.
47	46	110 approx.
31	28	110 „
51	53	51
41	45	45
51	53	110

From these determinations we passed to others upon blood withdrawn directly from the basilar vein.

In order to secure different degrees of venosity of the blood the arm was exposed, as has been done by previous workers, to different temperatures, either in one case hot water in others cold water or the temperature of the laboratory, whilst to secure the greatest degree of venosity the subject spent 15 minutes in the cold storage room prior to having his blood examined. The oxygen tension of the blood was measured by the bubble, whilst the percentage saturation of the same was measured with the differential apparatus.

The dissociation curve of blood of gradually increasing venosity has never actually been measured, but it has been calculated from theoretical data by Christiansen, Douglas and Haldane(5). The following data which we obtained agree very closely with their calculations.

Dissociation curve of blood from basilar vein (Nagahashi).

Percentage saturation	Oxygen pressure observed	Oxygen pressure read from C., D. and H.'s curve, corresponding to p.c. saturation
95	92	92–72 mm.
92		
74·8	50	49·4
74·8		
61	38	38 –37·4
59·2		
61·6	38	38·4–36·5
57·5		
15·8	14	16·7–15·2
13·7		

The above figures as well as confirming Christiansen, Douglas and Haldane's curve also confirm Meakins and Davies(6) in their demonstration of the great range of venosity which may be obtained by exposing the skin of the arm to varying temperatures. Assuming the arterial blood to be 95 p.c. saturated and the venous blood to vary

between 93·5 and 15 p.c. saturation, the difference in percentage saturation between the arterial and venous bloods would vary between 1·5 and 80. If the metabolism of the skin were the same at the different temperatures—and there is at all events no reason to suppose that it is greater at the lower temperature—the above range of difference in the percentage saturations would correspond to something like a fifty-fold difference in the quantity of blood which traverses the skin.

The table illustrates the unsatisfactory nature of the attempt to determine the oxygen pressure from the percentage saturation when the latter is over 90 p.c. The difference between 92 p.c. and 95 p.c. saturation corresponds to a difference of 72 and 92 mm. respectively (or 20 mm.), whilst the difference between 61·6 and 57·5 p.c. saturation corresponds to a difference of less than 2 mm. oxygen pressure.

A sample of arterial blood drawn by direct arterial puncture gave an oxygen pressure of 99 mm., that in the alveolar air taken by Haldane and Priestley's method gave 101·8 mm. The slight difference has of course on a single determination no significance.

We wish to record our thanks to the Medical Research Council which bore a portion of the expense of the work and to Dr Bock who made the determinations of the percentage saturation of the blood.

Summary.

1. By the method described it is possible to measure the oxygen pressure in blood drawn directly from a vessel in man to within about 2 mm. on the average.

2. The dissociation curves for venous blood calculated by Christiansen, Douglas and Haldane has been confirmed experimentally.

3. The observations of Meakins and Davies on the great range of unsaturation of blood from the basilar vein when the arm is exposed to widely differing temperatures have also been confirmed. Our experiments indicate a fifty-fold alteration in the volume of blood traversing the skin of the arm per minute.

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THE EFFECT OF ADRENALIN UPON RESPIRATION.

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It has long been known that adrenalin, when it is injected into an animal, induces a profound change in respiration. In 1895, Oliver and Schafer(1) mentioned that extract of the suprarenal gland caused sometimes an arrest but more commonly a shallowing of respiration and that the effect was greater in the rabbit than in the dog. Four years later, Boruttau(2) described a progressive shortening of the inspiratory phase and a gradual prolongation of the expiratory phase, breathing coming to temporary standstill in full expiration. The effect was not altered by division of the vagi and on this account he believed that it was due to the direct action of adrenalin upon the medulla. Boruttau also showed that the change in respiration was produced when adrenalin raised an abnormally low blood-pressure to a normal height, that is to say the respiratory effect was not due to an abnormally high blood-pressure. In 1901, Langley(3) confirmed the observations of Oliver and Schafer and noted that the effect wore off upon repeated injections. Salvioli and Pezzolini(4) found that while in both the rabbit and the dog the respiratory movements became shallower, in the rabbit they became at the same time more rapid but in the dog less rapid. Kahn(5), like Boruttau, found the change to consist in a gradual shortening of the inspiratory and prolongation of the expiratory phase. With large doses short expiratory pauses occurred. He further found that adrenalin intensified apnœa produced in other ways, as for instance by stimulating the central end of the vagus. He suggested that the effect might possibly be due to centripetal impulses in sympathetic nerves set up in the abdominal viscera or in the heart and aorta, by the peripheral action of adrenalin. Nice, Rock and Courtright(6) compared the changes in blood-pressure caused by adrenalin with the changes in respiration. They found that both the fall of blood-pressure caused by a small dose of adrenalin and the rise of blood-pressure caused by a larger dose were

accompanied by a synchronous increase in respiration, sometimes preceded by a decrease. From these and other observations they concluded that the action of adrenalin was direct on the nerve-centres. The observations, however, do not distinguish between a direct action and an indirect action either by centripetal impulses or by changes in blood supply.

From the preceding account it will be seen that no serious attempt has been made to determine the cause of the respiratory variations produced by adrenalin. The common supposition that it has a direct action on the respiratory centre is purely hypothetical. My experiments were made to determine more exactly the method of action.

Methods. The animals used were rabbits and cats. They were given a preliminary dose of urethane except in one experiment the object of which was to show that the presence of this drug was in no way responsible for the result. C.E. mixture was used to complete anæsthesia. Respiration was recorded by connecting one limb of the Y-shaped trachea-tube to a thin rubber tambour, the other limb being open to the air. The advantage of this method is that it causes the least disturbance to the normal respiratory process. It does not record the duration of the inspiratory and expiratory contractions but this is of no importance for the object of my experiments.

The blood-pressure cannula was tied into one of the carotid or femoral arteries. The injections were made through cannulæ tied into the left or right jugular vein and sometimes into the distal end of the carotid or vertebral artery. It may be thought that when one carotid artery and one jugular vein are ligatured a disturbance of the circulation apparently so gross would show itself in a modification of the medullary functions. This, however, is not so, although the vein above the ligature becomes much distended with blood. Nor when one carotid artery is connected to the manometer is respiration influenced by clamping the carotid artery of the other side. All that happens is a slight rise in blood-pressure of mechanical origin. When the second artery is unclamped there is sometimes a slight transient increase in the depth of respiration which may possibly be due to stimulation of the medulla by blood which has stagnated in the part of the artery below the clamp.

The stock adrenalin used was the Parke-Davis 0·1 p.c. solution. The dose most commonly employed was 1 c.c. of ·01 p.c. adrenalin, sometimes 1 c.c. of ·005 p.c. or of ·1 p.c.

One further point requires mention. In my experience, contrary to that of MacWilliam (7), Ringer's solution to which sodium citrate has been added has itself an effect upon respiration. Respiration is deepened

and sometimes quickened. On this account it has been necessary to fill the tube connecting the artery and manometer with pure Ringer. Clotting is of course more liable to occur.

In the rabbit, when doses of adrenalin of the order of 1 c.c. of a ·01 p.c. solution are injected intravenously the changes in respiration are three in kind.

1. There is invariably a rapid diminution in depth usually proceeding to a pause of about 20 seconds duration. This pause occurs in expiration

Fig. 1. Rabbit. Adrenalin 1 c.c. of 0·1 p.c. intravenously. Respiration arrested then resumed at a faster rate.

Fig. 2. Rabbit. Adrenalin, same dose. Cheyne-Stokes respiration. The speed of the drum was changed during the record; rate of respiration is not altered.

Fig. 3. Cat. Adrenalin, same dose. Respiration much diminished in depth and frequency.

as is obvious from an inspection of the animal; as stated above, it is not shown in tracings by the method used.

2. The pause may be followed by typical Cheyne-Stokes respiration which gradually merges into respiration of regular type. The tendency to Cheyne-Stokes respiration seems to be largely a matter of individual susceptibility and it is more prone to occur with an already high blood-pressure and with large doses of adrenalin.

3. There is a well-marked tendency to change in the rate of respiration. It is not that respiration becomes progressively altered in rate as it diminishes in depth though such a change may occur. It is that when

respiration is resumed after the pause it is resumed at a rate quite different from the original, usually faster. If a second pause occurs in the course of Cheyne-Stokes respiration the rate after this pause may be again different. Usually the original rate is restored. Typical examples of these three modifications are shown in Figs. 1 and 2.

In the cat the results are more or less the same. With doses which are too small to produce the full effect respiration is diminished in depth and also in rate (see Fig. 3). This agrees with what Salvioli and Pezzolini found in the dog. Cheyne-Stokes respiration can also be induced but a larger dose is required than in the rabbit. A curious form

Fig. 4. Cat. Adrenalin 1 c.c. of ·01 p.c. intravenously. (*a*), (*b*) and (*c*) are continuous.
Fig. 5. Stimulation of central end of splanchnic nerve.

of such breathing is shown in Fig. 4. Towards the end of each period of respiration one deep breath is taken. The first six of these breaths are almost exactly equidistant from one another. Further, each period of respiration tends to be itself multiple. The same tracing also shows how the rate of respiration is changed.

There are three possible ways in which adrenalin may conceivably cause this diminution and temporary arrest of respiration. They have been investigated as follows:

Afferent impulses. If afferent impulses exist we should expect to find them arising from the two regions suggested by Kahn; the splanchnic

area, since it is here that adrenalin is most potent in other respects, and the heart and aorta since these organs seem to be put passively to the greatest strain. I have tested this hypothesis in two ways. In the first the central end of the splanchnic has been stimulated. When any effect at all is produced it has been in the direction of augmentation not diminution of respiration (see Fig. 5). In the second method the abdominal aorta has been ligatured above the origin of the cœliac axis and adrenalin injected below the ligature. This procedure has practically no effect upon respiration. As regards the heart and aorta my experiments are in agreement with those of previous observers in that the effect is not lessened by division of the vagi. I have found that in the rabbit this applies also to the depressors.

Fig. 6.

Fig. 7.

Fig. 6. Rabbit. Abdominal aorta compressed. Respiration is slightly decreased in depth, but not altered in rate.

Fig. 7. Cat. The same operation. Respiration is decreased in depth and in rate.

We may therefore conclude that adrenalin does not either directly or indirectly inhibit the respiratory centre by nervous impulses from the periphery.

Blood-pressure. Frequently, but by no means always, the change in respiration occurs concurrently and in a corresponding degree with the change in blood-pressure. This is particularly the case in regard to the recovery of the normal depth of respiration which usually occurs *pari passu* with the return to the normal in blood-pressure. But even if such a relation were invariable it would not follow that the change in respiration was due to the change in blood-pressure, since effects upon the medulla and upon the arterioles in the rest of the body, though independent, would be likely to take approximately the same time to develop, become established and die away. Such correspondence would be still more

likely if the action upon the respiratory centre were the result of constriction of the blood vessels in the medulla.

That, apart from the injection of adrenalin, a relation exists between respiration and blood-pressure has been shown by Guthrie and Pike(8). These observers have found that when the blood-pressure in the carotid artery is raised by occluding the dorsal or abdominal aorta respiration is slightly quickened but diminished in amplitude. As regards the change in amplitude, I am in agreement with Guthrie and Pike. Fig. 6 shows that when in the rabbit the abdominal aorta is kinked by pulling upon a loop of string tied loosely round it, respiration is definitely diminished. There is, however, no change in rate. When the same procedure is applied to the cat (Fig. 7) the diminution in the depth of respiration is more striking and is accompanied by considerable diminution in rate.

It is clear therefore that the rise in blood-pressure itself is at least a contributory cause of the change in respiration which occurs when adrenalin is injected. The first question is whether it is the sole cause. Comparison of Figs. 3 and 7 which are taken from the same animal suggests that for an approximately similar rise in pressure a greater change in respiration is produced by injection of adrenalin than by occlusion of the abdominal aorta. Experiments were then made to determine what changes adrenalin produces when there is little or no rise of blood-pressure. In order to eliminate as far as possible any change in blood-pressure I have made use of the compensator shown in Fig. 8. It is essentially a modification of the mercury valve described by Bayliss. A glass T-piece *C* connects with an upright glass-tube *A* of about 2 metres length, with a bottle *B*, the height of which can be adjusted, and with a coil of tubing the other end of which is connected with the abdominal aorta. The coil of tubing is inserted in a basin of water at body temperature. The passages are clamped as shown at *E*, *F* and *G*. The clamp *G* being closed and *E* and *F* open the height of the fluid in the tube *A* is adjusted

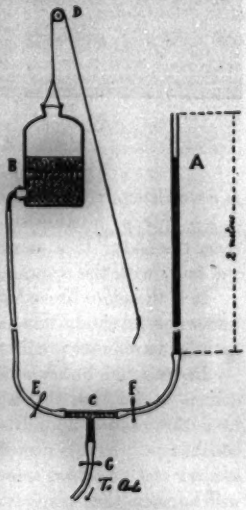

Fig. 8. Blood-pressure compensator.

at a level above the cannula in the abdominal aorta corresponding approximately to the pressure as indicated in the mercury manometer which is inserted into the carotid artery. *E* is then closed, *F* and *G* opened and the clamp released from the artery. The column of fluid in *A* having adjusted itself, the bottle *B* is raised or lowered so that the fluid in it is at the same level as the fluid in *A*. The clamp *E* is then opened.

When adrenalin or any other pressor substance is injected the resulting rise of blood-pressure drives blood into the apparatus, and since the area of surface of the bottle *B* is comparatively large there is practically no change in blood-pressure. As the effect of the adrenalin which remains in the circulation wears off the blood returns into the body. Fig. 9 shows the effect of injecting 1 c.c. of ·01 p.c. adrenalin. The rise in blood-pressure is very slight and soon passes off. The change in respiration, though it may be not quite so intense as it is without the compensator,

Fig. 9. Rabbit. Adrenalin 1 c.c. ·01 p.c. intravenously with the compensator described in the text.

is nevertheless very marked and it is always much more prolonged. It is less intense because so much of the adrenalin escapes temporarily from the body. It is more prolonged because adrenalin is returning to the body with the returning blood.

It is therefore proved that the rise in blood-pressure, though it may under normal circumstances contribute to the change in respiration, plays no large part in its production.

Direct action on the medulla. By a process of exclusion then we are driven to the view that adrenalin produces its effect upon respiration by a direct action upon the medulla. With the object of confirming this conclusion I have injected adrenalin into the peripheral end of the carotid and vertebral artery. A typical result is shown in Fig. 10. It will be seen that respiration begins to be diminished very definitely before the blood-pressure begins to rise. It must be admitted, however, that the change in respiration does not set in as early as might be expected.

When we consider how adrenalin stops respiration by an action on the medulla two possibilities present themselves; first that it causes profound vaso-constriction of the blood vessels of the medulla which leads to paralysis of the centres owing to oxygen want or accumulation of metabolites; secondly that it exerts a specific paralysing action upon the cells composing the respiratory centre.

In order to test the first possibility, I have tried the effect upon respiration of sudden anæmia of the medulla produced in other ways.

1. The animal is bled rapidly by opening the dorsal aorta. The result is shown in Fig. 11. Accompanying the sudden fall in blood-pressure

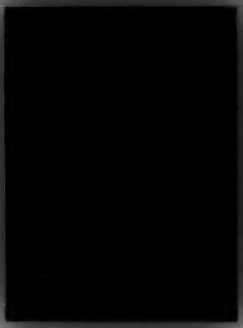

Fig. 10.

Fig. 11.

Fig. 10. Rabbit. Adrenalin, 1 c.c. ·002 p.c. injected into the distal end of the carotid artery. Respiration diminishes before blood-pressure rises.

Fig. 11. Rabbit. Sudden bleeding from the abdominal aorta.

there is an immediate stoppage of respiration, which lasts until the final spasms set in. At first sight, this fact seems to be contradictory to the statement made above that respiration is diminished by rise and increased by fall in blood-pressure. The two cases are, however, not really comparable for while there we were dealing with a partial and relatively slow change in pressure, we are here concerned with a complete and abrupt fall which must result in a complete and sudden anæmia of the brain.

2. A similar effect upon respiration can be produced by clamping the two carotid and two subclavian arteries (the latter below the origin of the vertebrals). A typical result is shown in Fig. 12. This experiment

has, of course, been performed by numerous workers in the past all of whom have found that ligature of all four vessels stops respiration. But the point which I want to emphasise is that the apnœa is sudden in onset.

It may therefore be concluded that the apnœa produced by adrenalin can be sufficiently explained by assuming that the substance causes a well-marked vaso-constriction of the vessels of the medulla. The centre is paralysed by want of oxygen or accumulation of metabolites. This does not preclude the possibility of a specific action upon the nerve-cells, but it renders such an explanation unnecessary.

Further experiments are in progress with the view to determining whether respiration can be modified by sympathetic stimulation.

Fig. 12. Rabbit. Clamping in succession the carotid and vertebral arteries.

Conclusions.

1. In anæsthetised cats and rabbits, adrenalin in large doses besides causing diminution and temporary arrest of respiration, causes alteration in rate and may cause Cheyne-Stokes respiration.

2. The diminution and arrest occur when there is no change in blood-pressure and are mainly central in origin.

3. They are caused by sudden anæmia of the respiratory centre due to vaso-constriction.

4. There is no evidence that adrenalin causes afferent nervous impulses from the periphery.

I wish to thank Prof. Langley, Mr Barcroft and Dr Dixon for their kindly interest and helpful suggestions.

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THE ACTION OF CARBON DIOXIDE ON SALT AND WATER DISTRIBUTION IN BLOOD. By GENKO MUKAI, M.D.

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The osmotic behaviour of the red blood corpuscles has been much debated since Hamburger (1883) pointed out that the amount of water in the corpuscles was determined by the law of osmotic pressure. Many investigators have carried out experiments by the most diverse methods (hæmolysis, hematocrite and cryoscopic method) and have supported his view, as long as the semipermeability of the membrane was preserved. In 1894, Limbeck(1) found that the corpuscles absorbed water from plasma, when carbon dioxide was passed through a sample of blood. If the same law holds good also in this case, the osmotic pressure of the corpuscles must increase more than that of plasma. The results so far obtained by the Δ determination render this doubtful, and at the suggestion of Prof. Bayliss I have investigated the question.

All experiments were performed with defibrinated dog's blood, which was drawn from a cannula in the carotid artery of a dog under chloroform and ether. Special care was taken to use the blood as soon as possible.

A. Changes produced in blood by carbon dioxide.

The distribution of salts and water. It is well known that carbon dioxide has a great effect upon the osmotic pressure of blood. The mere addition of CO_2 raises the osmotic pressure considerably more than the molar concentration of the added substance would explain. This effect is generally understood as a result of a complex reaction of CO_2 with the salts of the blood. As they are separated by a semipermeable membrane, corpuscles contain different salts from those of plasma, both in quality and quantity, which is responsible for the different osmotic effects of CO_2 on both sides of the membrane. Experiments were made therefore to determine the distribution of salts and water in the blood at various tensions of CO_2.

Exp. I. Dog's defibrinated blood was divided into two portions. Sample *A* was exposed to the air. Sample *B* was equilibrated with CO_2 in a large flask which contained almost pure CO_2. The following were determined:

(1) The volume percentage of the corpuscles. The blood samples were centrifuged beneath liquid paraffin until the reading became constant.

(2) The solid. A known volume of blood and of serum was weighed, dried at about 100°–110° C. until the weight became constant.

(3) Ash as sulphate. The same quantity of diluted sulphuric acid was added to 4 c.c. of the samples and then dried and ashed to constant weight.

(4) Chlorine content. After removing the protein from blood and serum by my method[1], the chlorine contents were determined by Volhard's method.

			4 c.c. serum		100 c.c. blood*			
					Before CO_2 treatment		After CO_2 treatment	
Sample		4 c.c. blood	Before CO_2 treatment	After CO_2 treatment	Serum	Corpuscles	Serum	Corpuscles
I.	Volume c.c.	—	—	—	58·7	41·3	54·6	45·4
	Weight gm.	4·105	4·024	4·032	—	—	—	—
	Water „	3·364	3·748	3·733	—	—	—	—
	Solid „	0·741	0·276	0·299	—	—	—	—
	Ash „	0·034	0·036	0·038	0·528	0·322	0·519	0·331
	NaCl „	0·022	0·026	0·023	0·382	0·168	0·314	0·236
II.	Volume c.c.	—	—	—	54·3	45·7	50·3	49·7
	Weight gm.	4·036	4·042	4·041	—	—	—	—
	Water „	3·270	3·732	3·706	—	—	—	—
	Solid „	0·766	0·310	0·335	—	—	—	—
	Ash „	0·038	0·044	0·047	0·597	0·353	0·591	0·359
	NaCl „	0·020	0·024	0·021	0·326	0·174	0·264	0·236
III.	Volume c.c.	—	—	—	44·9	55·1	39	61
	Weight gm.	4·104	4·042	4·038	—	—	—	—
	Water „	3·187	3·732	3·689	—	—	—	—
	Solid „	0·917	0·310	0·350	—	—	—	—
	Ash „	0·041	0·045	0·049	0·505	0·520	0·478	0·547
	NaCl „	0·020	0·026	0·021	0·292	0·208	0·205	0·295

* The total amounts of NaCl and ash present in serum and corpuscles were calculated from the percentage of both components of blood.

It will be noted that under the influence of CO_2: (1) The corpuscles increase in size by taking up water from serum. (2) The percentages of the solid and ash increase in serum. (3) The total amount of ash as sulphate remains the same in serum as a whole, when allowance is made for the loss of water, which shows clearly that cations do not pass from corpuscles into serum, and that there is no interchange of base between them. This result is identical with that of Gürber(2) and Hoeber(3). (4) As Hamburger(4) pointed out, the total amount, as well as concentration, of chlorine decrease in serum which must have entered into corpuscles. These changes have been proved to be reversible and parallel in response to the variations of CO_2 tension (Hamburger(8), Limbeck(5), Haggard, Howard and Y. Henderson(6), Fridericia(7)).

[1] This will be published shortly in the *Biochemical Journal*.

Exp. II. The dog's blood was divided into several portions. After exposing it to the given tension of CO_2, each sample was centrifuged under liquid paraffin and then the volume percentage of corpuscles, the chlorine concentration of serum and the percentage of the solid in serum were determined.

The effect of increased CO_2 tension upon the salt and water distribution in blood.

	4 c.c. of serum		In serum		
CO_2 tension mm.	Weight gm.	Solid gm.	% of solid	% of NaCl	Vol. % of corpuscles
(1) Exposed to the air	4·034	0·324	8·04	0·639	53·4
(2) 143	4·048	0·351	8·66	0·602	57·0
(3) 225	4·052	0·355	8·76	0·564	57·5
(4) 263	4·036	0·353	8·75	0·554	57·5
(5) 309	4·047	0·356	8·79	0·545	58·0
(6) 347	4·057	0·360	8·87	0·489	58·5

The capacity of serum to carry CO_2. There is a close relation between the salts and the CO_2 contents of serum as the following experiments show.

Exp. III. Defibrinated dog's blood was divided into two portions, which were treated as follows: Sample *A* (blood). Sample *B* (serum). The serum was obtained by centrifuging. Both samples were divided again into six portions and each was equilibrated respectively with the air and air containing 75, 112, 153, 186 and 223 mm. of CO_2 at room temperature. All portions of sample *A* were then centrifuged under liquid paraffin and serum was separated. The amount of CO_2 in the serum was determined by Van Slyke's method.

The effect of increased CO_2 tension upon the CO_2 carrying power of serum.

c.c. CO_2 content in 100 c.c. of serum

CO_2 mm.	Serum sample *A*	Serum sample *B*	Difference
Exposed to the air	29*	29*	0
75	63	41	+22
112	70	47	+23
153	72	49	+23
186	73·5	49·5	+24
223	75	51	+24

* After exposing to the air, serum of sample *B* was obtained.

Exp. IV. The same blood was used. The blood was exposed to the air containing CO_2 at 186 mm., and was divided into two portions. Sample *A* (blood). Sample *B* (serum). The serum was separated from corpuscles. Both samples were divided again into three portions and then were equilibrated with CO_2 at various decreased tensions. Sample *B'* was a portion of the serum of sample *A*, which was separated at 153 mm. of CO_2, and was treated like sample *B*.

The effect of decreased CO_2 tension upon the CO_2 carrying power of serum.

c.c. CO_2 content in 100 c.c. of serum

CO_2 mm.	Serum sample *A**	Serum sample *B*	Serum sample *B'*
186	73	73	—
153	72	—	72
112	—	72	—
75	63	68	66
40	—	—	49

* Serum of sample *A* was obtained after exposing to the decreased tension of CO_2.

From these data we see the influence of corpuscles on the capacity of serum to carry CO_2. Both samples *A* and *B* change their CO_2 contents in accordance with variations of tension; but the former is more liable to such change than the latter. This different behaviour of serum is attributed to the varying distribution of salts and water in blood under CO_2 treatment. As long as serum is in contact with corpuscles and these alterations are reversible, serum contains a definite quantity of CO_2 at a given tension, no matter whether the tension is decreased or raised to that degree. (Compare sample *A* in Exp. III with sample *A* in Exp. IV.) After separation, serum is free from the influence of the corpuscles and carries CO_2 by its own capacity.

At zero tension of CO_2 there is practically no trace of it in blood, but the serum, which has been previously separated from corpuscles, still contains a certain quantity. This remainder of CO_2 is found to be alkali carbonate and variable in quantity in proportion to the tension at which the serum is obtained. The fact shows clearly that there is an actual change in the total amount of diffusible alkali in serum as the result of treating blood with CO_2 (Loewy and Zuntz(9), Hamburger(10)). It might be explained by an interchange of either acid or basic radicals between corpuscles and serum. While there is no evidence of alkali interchange, the chlorine passes to and fro between them in response to variation of CO_2 tension. Thus alkali of neutral salts (NaCl, KCl, etc.) is released in serum to form alkali bicarbonate, if the tension is raised, and *vice versa*. Gürber(11), Van Slyke and Cullen(12) and Fridericia(7) have proved by chloride analysis that the amount of HCl formed from NaCl and passing into corpuscles corresponds to the greater part of the amounts of alkali bicarbonate produced in plasma. Thus the influence of corpuscles upon the capacity to carry CO_2 of serum may be regarded as a result of changed distribution of anions in blood.

The distribution of CO_2 *between corpuscles and serum.* Carbon dioxide in blood is approximately equally distributed between corpuscles and serum at every tension of CO_2. There is, however, a small difference between them.

Exp. V. Defibrinated dog's blood was equilibrated with CO_2 at various tensions.

	CO_2 content in 100 c.c. of blood and serum		
CO_2 mm.	Blood c.c.	Serum c.c.	Difference
48	59	63	−4
100	68	72·5	−4·5
122	70	74	−4
171	81	80	+1
179	85	81	+4
247	92	84	+8

At low tension of CO_2, blood carries less of it than the corresponding serum. The difference between them becomes smaller and smaller until they contain the same quantity at 170 mm. of CO_2. At tension higher than 170, blood contains more CO_2 than its serum. This result can be explained by the unequal distribution of CO_2 between corpuscles and serum at each tension except 170 mm. In other words, the increase of CO_2 content in corpuscles is always greater than that in serum, although both increase in proportion to the CO_2 tension.

If the tension of CO_2 is decreased, this relation between corpuscles and serum becomes the reverse, the corpuscles lose CO_2 more than serum.

It is of interest to compare the effects of the decreased CO_2 tension upon the blood and the separated serum. The serum which has been separated from corpuscles loses less CO_2 than that which has not (see Exp. IV). Blood is most sensitive to the alternation of CO_2 tension and loses CO_2 in the highest degree.

These unequal effects of decreased tension must be borne in mind when determining the freezing point of blood. The higher the tension of CO_2, at which the serum is separated from corpuscles, the greater the difference of escaping CO_2 from blood and separated serum.

The mechanism by which the corpuscles carry CO_2. There is a remarkable fact in Exp. V which shows that the quantity of CO_2 in corpuscles changes in proportion to the variation of the CO_2 tension. This would seem to contradict the result, which might be expected from the interchange of acid radicals. The explanation of this is that proteins are amphoteric bodies and that the isoelectric points of hæmoglobin, serum globulin and serum albumin are different. Thus hæmoglobin releases all its alkali at $C_H 1{\cdot}8 \cdot 10^{-7}$, while the serum proteins set free only a small part of it. On the other hand, the corpuscles contain about 30 p.c. of hæmoglobin, which is a very high concentration compared with the protein in serum (about 8 p.c.). As the quantity of the newly produced bicarbonate depends on the amount of alkali-protein, and the rate of its increase on the isoelectric point of protein, the corpuscles have a stronger and quicker capacity to form bicarbonate than serum (Zuntz(15), Hamburger(10)). If carbon dioxide is led through the blood, these capacities of both corpuscles and serum will undergo a change.

By means of acid radical interchange, the serum gets more available alkali to combine with CO_2, while the corpuscles lose it in just the same degree, *i.e.* corpuscles lose just what the serum gains, and the CO_2 carrying power of the blood remains uninfluenced by this interchange of acid radicals. The relation can be seen in the following experiment.

Exp. VI. Dog's blood was divided into two portions. Sample *A* (not hæmolyzed). Sample *B* (hæmolyzed by freezing and thawing). Both samples were equilibrated with CO_2 at various tensions and analyzed for CO_2 content.

	c.c. CO_2 content in 100 c.c. of		
CO_2 mm.	Hæmolyzed blood	Not hæmolyzed blood	Difference
112	66·5	66·5	0
153	71	70	+1
186	75	75	0
223	80·5	80	+0·5

* Blood was hæmolyzed and then exposed to CO_2.

This shows also that (1) hæmolysis itself has no effect upon the amount of CO_2 in whole blood, (2) the chlorine seems to be an agent, which regulates the distribution of CO_2, C_H and the osmotic pressure of blood, so that they are approximately equal on both sides of the corpuscles.

B. The increase of the molar concentration of blood under CO_2 treatment.

When blood is treated with CO_2, both corpuscles and plasma increase in osmotic pressure. If CO_2 is led through a sample of plasma, which has been separated from corpuscles, the increase of osmotic pressure is decidedly less than that which has not.

Exp. VII. Defibrinated dog's blood was divided into two portions. From one part, serum was obtained by centrifuging. Sample *A* (whole blood). Sample *B* (serum). Both samples were exposed to the air containing 40, 510, 600 mm. of CO_2 at room temperature and then the Δ of serum determined.

CO_2 mm.	Δ of the serum of *A* °C.	Δ of the serum of *B* °C.
40	−0·605	−0·585
510	−0·675	−0·630
600	−0·760	−0·700

The difference of osmotic pressure is due to the alteration of the capacity to carry CO_2 of serum. From this it is evident that a close relation exists between the osmotic pressure and the CO_2 carrying power —the amount of CO_2—of blood. On the other hand, it has been proved that corpuscles take up CO_2 more than the corresponding serum at every tension of CO_2 and absorb water from serum. If CO_2 were in one form in blood, then the explanation of osmosis into corpuscles would be quite clear.

Corpuscles are free to change in size by taking and giving up water from the surrounding fluid. Thus corpuscles are supposed always to have the same osmotic pressure with surroundings. According to the osmotic

theory, the increase of water in the corpuscles under CO_2 treatment must be the result of increased internal concentration of substances to which the membrane is impermeable.

The freezing point determination seems to be the only method which gives a direct experimental answer to the enquiry as to whether the osmotic pressures are equal on both sides of the corpuscles, *i.e.* whether the law of osmosis is perfectly applicable to blood corpuscles. Hamburger(16) carried out such measurements and obtained results which showed that the corpuscles had a less osmotic pressure than the plasma, both in the normal condition and in the case of blood treated with CO_2. He insisted, however, that the law of osmosis was applicable and ascribed the deviations to the adsorptive phenomenon of crystalloids after bæmolysis. Some authors assume the swelling pressure of the protein, which compensates the osmotic negative pressure of the corpuscles, and some go so far as to state that the regulation of the amount of water between corpuscles and plasma is solely determined by the protein and not by the salts. Such ideas are contradicted by the following experiments.

Cryoscopic method. By the freezing and thawing method I hæmolyzed the blood and repeated the experiment of Hamburger.

Exp VIII. After exposing the blood to the air for one hour, I determined the Δ of both hæmolyzed blood and serum with Beckmann's apparatus.

The Δ of normal blood

Sample	Δ of hæmolyzed blood ° C.	Δ of serum ° C.	Difference ° C.
1	−0·580	−0·587	+0·007
2	−0·605	−0·613	+0·008
3	−0·581	−0·595	+0·014
4	−0·592	−0·597	+0·005
5	−0·582	−0·593	+0·011
6	−0·573	−0·585	+0·012

Exp. IX. After exposing the blood to carbon dioxide, I divided it into two parts: One part was hæmolyzed under liquid paraffin. The other was centrifuged under liquid paraffin, which separated serum from corpuscles. The Δ of both hæmolyzed blood and serum were determined.

The Δ of blood treated with CO_2

Sample	Δ of hæmolyzed blood ° C.	Δ of serum ° C.	Difference ° C.
1	−0·655	−0·708	+0·053
2	−0·750	−0·770	+0·020
3	−0·72	−0·76	+0·04
4	−0·67	−0·73	+0·06
5	−0·63	−0·69	+0·06
6	−0·725	−0·787	+0·062
7	−0·674	−0·715	+0·041
8	−0·716	−0·767	+0·051

These results are identical with those of Hamburger.

There are, however, some possible sources of error in these experiments:

1. Hamburger attributed the difference of the Δ between hæmolyzed blood and serum to an adsorption of crystalloids by the colloids of the corpuscles. This might be so, but he did not satisfactorily explain why the osmotic difference was so great in the case of blood treated with CO_2.

2. There is an opportunity of CO_2 escaping from the fluid, which changes the Δ of it. The first reading of the freezing point is always lower than the second. The difference between the first and second readings is found to be enormous when the determination is made after CO_2 treatment. But serum, as a rule, is less liable to change its Δ than hæmolyzed blood. Hamburger(17) has pointed out that the Δ of serum can be determined without separation from corpuscles unless it contains much CO_2. In the latter case it must be separated from the corpuscles, otherwise the Δ of this serum reads less than it ought. These facts seem to coincide with what I noticed in Exps. IV and V. By exposure to the air, the blood loses very quickly a considerable amount of CO_2, while the separated serum only loses a small quantity. The error caused by escaping CO_2 is magnified by the stirring and the long exposure, which cannot be avoided in the cryoscopic method. The higher the concentration of CO_2, the greater the error.

As far as the amount of CO_2 is concerned, hæmolysis has no effect upon the blood, but the tension of CO_2 has great effect. For this reason I decided to adopt another method of determining molar concentration, which prevents the escape of CO_2 from the fluid.

Barger's microscopical method. In 1904 Barger(18) published a method, which seems most suitable for this purpose: A solution of unknown molar concentration is compared with a standard solution. A series of drops are collected from the two solutions alternately into a glass capillary tube. After sealing both ends of tube, the length of the drops is measured with a micrometer under the microscope. If the vapour pressure is equal in both solutions, they have the same molecular concentration and there is no change in the length of the drops.

In order to use this method with blood, some special conditions are necessary. Since hæmoglobin is apt to crystallize out, the blood should be diluted to a known degree with its own serum. The standard solution must have the same hydrogen-ion concentration and the same tension of carbon dioxide as the blood or serum. Otherwise the latter will lose

carbon dioxide and its osmotic pressure fall. The blood in the capillary is hæmolyzed by freezing and thawing after sealing.

Two methods were used. In the first, alternate drops of blood and corresponding serum were compared. In the second, the blood and the serum were separately compared with solutions of sodium chloride of known strength. The second method is exposed to a slight error owing to the possibility of unequal loss of CO_2 from blood and from serum.

All samples of blood and serum were kept under liquid paraffin. Directly before use, I took out a small portion of them in crucibles, taking care to avoid mixing them with paraffin. It was reasonable to suppose that some carbon dioxide escaped from the fluid during the manipulation. To restrict these errors to the minimum, I took drops of equal length as quickly as I could and tried to leave equal air spaces between them.

Exp. X. Normal blood. The volume change of drops in Barger's method, at 15° C., in the first 24 hours.

(Ocular micrometer 0·1 mm. No. 2 and Leitz objective No. 2.)

By the first method					Standard NaCl solution	By the second method: (*a*) The blood was compared					By the second method: (*b*) The serum was compared				
a	*b*	*a*	*b*	*a*	p.c.	*a*	*s*	*a*	*s*	*a*	*b*	*s*	*b*	*s*	*b*
					0·934	+7	−6	+3	−3	+4	+4	−3	+3	−4	+2
±0	±0	−1	±0	±0	0·942	+5	−3	+3	−3	+1	+2	−1	+2	±0	+2
					0·952	+1	−2	+2	−1	+2	+2	−1	+3	±0	+3
−1	±0	±0	±0	±0	0·961	±0	±0	±0	−1	+1	−1	+2	±0	+2	+1
					0·971	−2	+1	−3	±0	−1	−2	+3	−1	+2	−2
Sample 1. Result $a=b$						$a=0{\cdot}961$ % NaCl					$b=0{\cdot}961$ % NaCl				
					1·00	−3	+2	−3	+2	−2	−3	+2	−3	+2	−1
+1	−1	±0	±0	±0	0·990	−1	−1	±0	−1	+1	+1	±0	±0	−2	+1
					0·982	+1	±0	+2	+2	+2	−1	−1	+3	+2	+2
−1	−1	−1	−1	±0	0·971	+3	−4	+5	−1	+3	+13	−9	+12	−12	+15
					0·963	+3	−6	+3	−4	+2	+4	−5	+5	−7	+2
Sample 2. Result $a=b$						$0{\cdot}990>a>0{\cdot}982$ %					$0{\cdot}990>b>0{\cdot}982$ %				

Many other experiments gave the same results.

a indicates blood which is diluted with the same quantity of serum.

b indicates serum.

s indicates standard NaCl solution. The length is indicated in one-tenth of one degree.

These data show that: (1) The molar concentration of the normal blood is the same inside and outside the cells. (2) The difference of molar concentration, which corresponds to the Δ of 0·005° C., can be easily distinguished by this method.

Exp. XI. Blood was treated with CO_2. After establishing equilibrium I separated the serum from corpuscles and measured the molar concentration.

The volume change of drops in Barger's method, at 15° C., in the first 24 hours.

(Ocular micrometer 0·1 mm. No. 2 and Leitz objective No. 2.)

By the first method					Standard NaCl solution	By the second method: (*a*) The blood was compared					(*b*) The serum was compared				
a	*b*	*a*	*b*	*a*	p.c.	*a*	*s*	*a*	*s*	*a*	*b*	*s*	*b*	*s*	*b*
					1·080	−2	+2	−4	+5	−3	−2	+3	+1	+5	−3
+1	±0	−1	−1	±0	1·061	−2	+1	−2	+4	±0	−2	+4	−3	+1	±0
					1·052	+5	−3	+6	−1	+5	+4	±0	+3	−2	+3
−1	±0	±0	±0	−1	1·043	+7	−4	+5	−2	+8	+7	−5	+6	−3	+2
					1·035	+6	−8	+12	−9	+3	+12	−7	+6	−8	+10
Sample 1. Result $a=b$						1·0614 % > *a* > 1·0522 %					1·0614 % > *b* > 1·0522 %				
+2	+2	±0	+2	+3	1·210	−5	+8	−1	+2	−4	−8	+7	−4	+5	−8
					1·090	−2	+3	−3	+1	−5	+5	+4	+1	+4	+4
±0	+3	+1	±0	±0	1·080	−3	−2	±0	+1	±0	+3	−2	+5	+6	+3
					1·069	+13	−2	+10	+4	+6	+5	+1	+5	+2	+6
+2	+2	+1	+3	+2	1·054	+8	−3	+7	+1	+5	+8	±0	+13	−1	+5
Sample 2. Result $a=b$						*a* = 1·080 %					1·090 % > *b* > 1·080 %				

Many other experiments gave the same results.

a indicates blood which is diluted with the same quantity of serum.
b indicates serum.
s indicates standard NaCl solution. The length is indicated in one-tenth of one degree.

There was no change in the length of the drops by the first method. The molar concentration of both blood and serum must be the same within the limits of accuracy of this method. A small difference between (*a*) and (*b*), which corresponds to the Δ of 0·005° C., was due partly to the unavoidable error caused by the escape of CO_2.

From this result it is safe to say that the molar concentration of the corpuscles is almost equal to that of the serum, at the most, the difference between them is below that which corresponds to a Δ of 0·01° C. The difference of the molar concentration between corpuscles and serum, which is obtained by the cryoscopic method, is due chiefly to the loss of CO_2.

C. Osmosis or diffusion after death?

It is said that the cells obey the osmotic law while the semipermeability of the membrane is preserved. As soon as this semipermeability is destroyed which occurs soon after death, the cells no longer follow this law and the salts diffuse through the membrane until their concentration becomes the same on both sides of the membrane. It may be asked whether the corpuscles were alive or not in the foregoing experiments. Unfortunately it is impossible to discriminate between the living

and dead corpuscles. For this purpose muscle is more suitable and I have made experiments on this.

Isolated frog's muscle seems to be isotonic with 0·7 or 0·75 p.c. of NaCl solution. It takes a long time to reach osmotic equilibrium and there is a tendency to accumulate metabolic products in the muscles, so that the muscles when active have a stronger osmotic pressure than when inactive. This was proved by experiment with fatigued muscle, which required more concentrated solution to keep its original weight. Otherwise this muscle took water from the surrounding medium and the weight reached a maximum about two to three hours later (Cooke[19], Fletcher[20]). For this reason I compared both sartorii of a frog and weighed them two or three hours later after immersing them in the fluid.

Exp. XII. M. sartorii of frogs were immersed in dog's blood serum, which was diluted with water so that the serum became isotonic with 0·7 p.c. NaCl solution. After weighing, I passed carbon dioxide through the serum, which contained one group (*a*) of these sartorii, and observed the change in weight and irritability to electric stimulant. Experiments were carried out by Overton's method (23).

Sample	Weight after immersing 3 hours in serum, gm.	D.	Percentage of increase in weight after (hours) 1	1½	2	3	4½	6	24
1 *a*	0·099	15	+6·6	+6·8	—	+6	+6·4	+6·4 D. 9	−3 D. 0
1 *b*	0·123	15	±0	±0	—	±0	±0	±0 D. 15	+3 D. 15
2 *a*	0·177	15	+5	+6	—	+7	+7·3	+7·3 D. 8	±0 D. 0
2 *b*	0·177	15	−1	−1·5	—	−1·5	−1	−1 D. 15	+5 D. 15
3 *a*	0·087	14	—	—	+7·6	+8	—	+8 D. 6	—
3 *b*	0·077	14	—	—	±0	±0	—	±0 D. 10	—

Muscle (*a*) was treated with CO_2.
Muscle (*b*) was, for the purpose of contrast, not so treated.
D. indicates the maximum distance (cm.) of an induction coil, at which the muscle contracted. D. 0 means dead. + means increase. − means decrease.

It is evident from this that muscles increase in weight after treatment with CO_2. The muscle lives after more than six hours of CO_2 treatment. Experiment in a solution, which contained 0·5 p.c. NaCl and 0·287 p.c. $NaHCO_3$ (isotonic with 0·7 p.c. NaCl solution), gave nearly the same result.

Exp. XIII. The same experiment was made in a 0·7 p.c. NaCl solution.

Sample	Weight after immersing 1 hour in NaCl sol., gm.	D.	Percentage of increase in weight after (hours) 1	4	24
1 *a*	0·095	11	+2	+2 D. 0	−10
1 *b*	0·083	14	+3	+4 D. 9	+23 D. 0
2 *a*	0·070	12	−3	−3 D. 0	−6
2 *b*	0·070	9	+3	+2 D. 9	+21 D. 0
3 *a*	0·074	13	+2	+2 D. 0	−13
3 *b*	0·075	9	+3	+2 D. 9	+20 D. 0

a treated with CO_2. D. as in Exp. XII.

The change in weight was so small in this experiment, that during life there was no appreciable effect of CO_2. After death there was a great difference between them; muscle treated with CO_2 decreased in weight, while the other increased greatly. This behaviour of dead muscle occurs to a greater or lesser degree in all experiments.

Exp. XIV. Muscle in 6·1 p.c. cane sugar solution (isotonic with 0·7 p.c. NaCl). The muscle died very quickly, after two hours it could not be contracted by electric stimulation.

Sample	Weight after immersing 1 hour in the solution, gm.	Percentage of increase in weight after (hours)			
		2½	3½	6	24
1 *a*	0·217	−3	−6	—	−12
1 *b*	0·207	+1	+1	—	+4
2 *a*	0·170	−5	−10	—	−15
2 *b*	0·160	−1	−1	—	−1
3 *a*	0·182	−2	−5	−8	−13
3 *b*	0·178	+1	+1·5	+1·5	+5

a treated with CO_2. D. as in Exp. XII.

It is evident from these experiments that: (1) the living muscles increase in weight by treatment with CO_2 and reach the maximum about two to three hours later; (2) as long as they are alive, they keep their weight in an isotonic salt solution; (3) soon after death there appears a change in their weight, although they are kept in the same solution.

This behaviour of the dead muscle finds its explanation in the physical property of muscle proteins. Muscle contains several kinds of protein gels. Some of them are characterized by their strong capacity for swelling and some of them by their power of spontaneous coagulation after death which is regarded as a phenomenon of dehydration. When a muscle is treated with CO_2, lactic acid is formed very rapidly and accumulates in it. On the one hand, this tends to cause some of the proteins to take up water, and thus the living muscles increase in weight for two to three hours. On the other hand, it tends to cause coagulation, *i.e.* dehydration. In a muscle treated with CO_2, the coagulation may occur almost immediately after death, so that soon after death there is a decrease of the osmotic pressure of muscle. A proof of this is that a muscle left in sugar solution till it is dead decreases in weight only after adding some quantity of acid (CO_2).

We have seen that muscle continues to live in serum over six hours after CO_2 treatment. I infer that the same is the case with blood corpuscles. The following conclusions may then be drawn: (1) the corpuscles increase in size by osmosis under CO_2 treatment, (2) the law of osmosis is perfectly applicable in the case of blood treated with CO_2,

i.e. water enters into the corpuscles until the osmotic pressure becomes equal on both sides of the membrane.

It may be argued against the result obtained by Barger's method that the corpuscles may change their osmotic concentration after death—hæmolysis—just like muscle. This may be possible to a certain extent, because there is a sudden production of lactic acid in corpuscles after death (Mellanby and Thomas(21)). Thus increased acidity may alter the degree of swelling of protein particles and may decompose any bicarbonate present in corpuscles. It is difficult to state how far this is actually the case. The main constituent of corpuscles—hæmoglobin—is a hydrophobic colloid which cannot change its degree of swelling. Of course other protein particles may change their swelling but this factor will be negligibly small on account of their low concentration. The factor of decomposing bicarbonate must be assumed in hæmolyzed blood. This error, if there is any, is included in the difference between corpuscles and serum obtained by Barger's method, but it is very small as shown in Exp. VI.

D. The cause of osmosis of the blood corpuscles under CO_2 treatment.

It remains to discuss the cause of osmosis of the corpuscles under CO_2 treatment. The foregoing experiments indicate that two factors come into play: (1) imbibition of colloid particles, and (2) change in salt distribution.

Imbibition of colloid particles. When blood is treated with CO_2, it increases in acidity. Consequently the hydrophilic colloids both in serum and corpuscles may change in swelling. The red blood corpuscles are a mixture of hydrophilic (protein of stroma and lecithin) and hydrophobic (hæmoglobin and cholesterin) colloids. Among them the protein of stroma is of great importance, which exists about 3–5 p.c. in corpuscles and is known as "fibrin like" in character. On the other hand, serum contains a very small quantity (6–7 p.c.) of hydrophilic colloids. Owing to their low concentration, the amount of water absorbed by these colloids must be small. If this factor plays any part in osmosis, it may be explained by the difference in the amount of water absorbed by colloids on both sides of the membrane, and it must be very small.

Change in salt distribution. Exp. V shows that the corpuscles take up CO_2 more than the corresponding serum at every tension of CO_2. Exps. I and II show that the chlorides also increase in the corpuscles in proportion to CO_2 tension. From these facts it is evident that the

essential cause of osmosis must be some compounds of CO_2 and chlorine. There are many forms of these compounds, but in general they can be divided into two groups; organic and inorganic. The former group is combined with protein and exists only in a reaction more acid than the isoelectric point of protein. The isoelectric point of hæmoglobin is about $C_H 1{\cdot}8.10^{-7}$ and can be easily reached by treating blood with 150 mm. of CO_2 (Hasselbalch(22)). On the contrary the isoelectric points of serum proteins have a very high C_H and are difficult to be reached. Thus at lower tension than 150 mm. of CO_2, blood combines with CO_2 and chlorine only in such forms as alkali carbonate, alkali bicarbonate, carbonic acid and alkali chlorides. Consequently the cause of osmosis below 150 mm. of CO_2 must be attributed to these salts and acid. If the tension of CO_2 is raised more than 150 mm., hæmoglobin can combine directly with CO_2 and chlorine, and the further increase of water in corpuscles is determined chiefly by the dissociation of these organic compounds.

Summary.

1. By treating blood with CO_2, the corpuscles increase in chlorine and water, but there is no evidence of cation interchange between corpuscles and serum.

2. At every tension of CO_2, corpuscles take up more CO_2 than the corresponding serum.

3. Carbon dioxide is carried partly by increased diffusible alkali and partly by protein in blood, the quantities of which depend solely on the tension of CO_2—C_H.

4. The error involved in the cryoscopic method is chiefly caused by the escape of CO_2.

5. By means of Barger's method, which is able to determine the molar concentration without losing CO_2, it has been proved that the law of osmosis is perfectly applicable in the case of blood treated with CO_2.

6. From the results obtained by the experiments with muscle, it may be inferred that the corpuscles are alive for more than six hours after CO_2 treatment and take up water by osmosis, not by diffusion.

7. The cause of osmosis is due to the increased CO_2 and chlorine in corpuscles.

I take this opportunity of thanking Prof. W. M. Bayliss, F.R.S. for his kindly help and advice during the progress of this work.

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THE MEASUREMENT OF THE OXYGEN CONTENT OF THE MIXED VENOUS BLOOD, AND OF THE VOLUME OF BLOOD CIRCULATING PER MINUTE.

By J. BARCROFT, F. J. W. ROUGHTON and R. SHOJI.

(From the Physiological Laboratory, Cambridge.)

The measurement of the oxygen in the mixed venous blood is of interest, since it forms the basis of the "Coefficient of Utilisation" (Krogh) and also because the deficit in oxygen between the venous blood and the arterial blood can be used as the basis of a method for the measurement of the minute volume. This principle was introduced by Fick in 1870 and has been used since by a great number of workers. The previous work will be found fully discussed in a paper by Liljestrand and Lindhard(1). In animals the composition of the mixed venous blood can be obtained by direct analysis, but in man it must be inferred from the alveolar air, which must in some way be brought into equilibrium with the venous blood. The application of the principle of Fick has been rendered more difficult as knowledge has advanced, for the work of Christiansen, Douglas and Haldane(2) has shown that the oxygen determinations are only correct if both the oxygen and carbonic acid in the alveolar air are in equilibrium with the venous blood. They have devised methods for the accomplishment of this, which have formed the subject of a demonstration to the Physiological Society.

The present research was undertaken in the hope that some more simple method might be forthcoming which would measure the oxygen content in the venous blood with sufficient accuracy for many purposes. The sources of error divide themselves into two main categories: (1) the technical errors contingent on inaccuracies in gas analysis and the production of some artificial equilibrium between the oxygen in the lung and that in the blood, (2) the physiological errors due to some upset of the circulation by the artificial process of respiration imposed by the experiment. In devising the present method we regarded errors of the latter type as the more seridus because they are so elusive. If it were possible to make the assumption that the composition of the general venous blood were constant the problem would be relatively simple, but

this assumption cannot safely be made, even when the body is not doing muscular work. In order therefore to get rid of the physiological errors as much as possible, we laid down certain conditions which our method should fulfil: (1) that the respiration should only be interrupted for five to seven seconds, the time necessary to transfer the mouth from any form of respiration apparatus to that in which the alveolar air is estimated and (2) that the estimation should be made in a single observation which would give the composition of the venous blood in the body at a given moment of time.

By breathing nitrogen in a way to be described presently an equilibrium may be produced between the mixed venous blood and the alveolar air in the lung. The partial pressure of oxygen in the alveolar air is then a measure of the partial pressure of oxygen in the mixed venous blood of the lung capillaries at the moment. This blood differs from the normal mixed venous blood inasmuch as it is depleted to some extent of carbonic acid. On this account the oxygen pressure is too low. By the application of an empirical correction an approximation to the proper oxygen pressure can be obtained which is as close to the correct value as other collateral errors would in any case admit of.

The method of respiration is as follows: a bag fitted with a tap and valves, as described by Douglas, is filled with nitrogen. The valves and tap are so disposed that the subject of the experiment can inspire at will either from the external air or the bag, but his expired air must in either case go into the outer atmosphere. He then goes through the following processes: (1) he clips his nose, (2) breathes through the valves, inspiring atmospheric air till he is satisfied that he is breathing normally, (3) makes a rather deep expiration, (4) turns the tap so that the next inspiration comes from the bag, (5) takes a rather deep inspiration from the bag and then breathes normally for several respirations (four or five according to the rate of respiration), (6) turns the tap on the bag so as to shut it. (7) With his mouth closed and taking no further inspiration he turns to a Haldane gas analysis apparatus which is in readiness, fitted with an alveolar air tube and takes a sample of his alveolar air. (Process (7) should take about six seconds.) The person who is conducting the experiment takes the time with a stop-watch between operations (4) and (6). This is known as the "interval of nitrogen respiration." Our convention is not to include in it the time taken over operation (7). The actual time therefore occupied in the establishment of the equilibrium is about six seconds longer than the "interval of nitrogen respiration."

Evidence that equilibrium is established between the air in the lung and the blood in its capillaries.

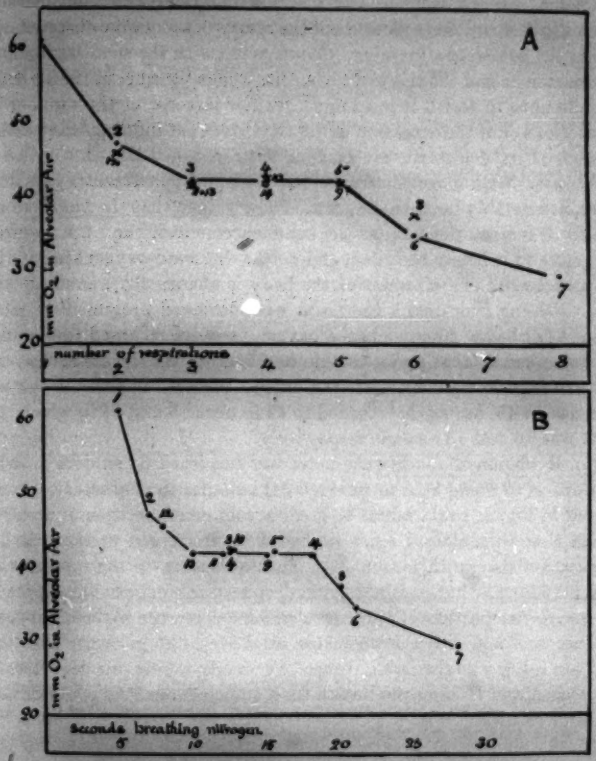

Fig. 1. *A.* ordinate = alveolar oxygen pressure.
abscissa = number of respirations in the interval of nitrogen respiration (hereafter referred to as I.N.R.).

B. ordinate as in *A*.
abscissa = duration in seconds of I.N.R.

Numerals attached to the points indicate the order in sequence in which the determinations were made. The two graphs represent the same set of determinations.

(*a*) The first point we would urge is that within considerable limits the composition of the alveolar air is independent of either the length

of the "interval of nitrogen respiration" or of the number of respirations included in it. The results plotted graphically in the figure illustrate this point.

In Fig. 1 *A* and *B* are plotted out the results of successive observations during the course of a morning. Commencing with the smallest number of respirations and the shortest I.N.R. the oxygen pressure in the alveolar air falls until in test 3 it is 42 mm.; further increase of the number of respirations or of the extension of the I.N.R. does not influence the partial pressure of oxygen in the alveolar air until in test 6 the I.N.R. reaches 21 seconds. With longer intervals (test 7) the oxygen pressure falls still lower, presumably because the I.N.R. is now so long that the blood which already traversed the lung at the commencement of the I.N.R. returns once more to it before the close, and not having been oxygenated at the commencement of its circuit of the body is abnormally venous at the close. Passing from test 7 the I.N.R. was shortened progressively until when it fell below 20 seconds the oxygen pressure returned to 42 mm., and remained at that figure (within half a mm.) till the I.N.R. was cut down to four to eight seconds; the oxygen pressure in the alveolar air then rose to 45, but again returned to 42 in tests 13 and 14 in which the I.N.R. was 10 and 18 seconds respectively.

(*b*) Evidence of another character was furnished by subject S., who in addition to being able to produce data similar in character to those plotted in Fig. 1, could inhale so great a volume of nitrogen at a single breath that with short I.N.R.'s he had a lower oxygen pressure in his alveolar air than with longer ones. Thus according to the quantity of nitrogen which he inspired in the first deep respiration he could approach the horizontal portion of the curve which relates the alveolar oxygen pressure to I.N.R. either from below or above—but in either case the ultimate result was the same. Indeed by simply taking one deep breath of nitrogen and holding the breath for a sufficient time he could obtain the same result (cp. Fig. 2 *A*).

In this figure dots represent determinations of the oxygen in the alveolar air produced by the procedure described above, whilst the crosses were determinations after a different procedure, namely: A maximal expiration was made, then a maximal inspiration of nitrogen —the breath was held for a length of time expressed along the abscissa at the end of which time the alveolar air was taken and analysed. The ultimate identity in position of the plateaux on the two curves, one approached from above, the other approached from below, seems to leave no doubt that in each case when the plateau was reached, the alveolar

air and the blood in the lung capillaries were in equilibrium and therefore that the partial pressure of oxygen in the alveolar air was the measure of that in the blood.

Correspondence between the partial pressure of oxygen of the alveoli, and that of mixed venous blood.

In making the analyses of the alveolar air, the partial pressure of CO_2 was estimated in every case, as well as that of oxygen. In the plateau region the carbonic acid pressure was very regularly about

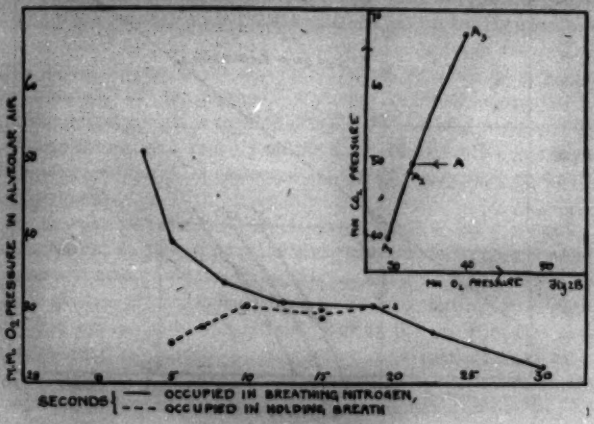

Fig. 2 *A* and *B* (inset).

40 mm.—a value which corresponds to that for normal alveolar air, but not that for normal mixed venous blood. Clearly then the CO_2 pressure is below what it should be and therefore the CO_2 pressure in the lung capillaries is below what it should be; but if that is so, it follows that there is an error in the oxygen pressure, for if the CO_2 pressure be raised, the oxygen pressure in the venous blood will be raised, and therefore an equilibrium will be struck with a higher pressure of oxygen in the alveolar air. The error involved was investigated on Shoji. Four determinations were made with no CO_2 in the inspired air, a fifth with about 5 p.c. CO_2, a sixth with a further addition of CO_2. The oxygen and CO_2 in the alveolar air were as follows:

TABLE I.

Experiment	(1)	(2)	(3)	(4)	(5)	(6)	Mean of (1), (2), (3), (4)
Pressure of gases in alveolar air in mm. O_2	27·8	27·2	29·8	30·4	32·2	39·6	29·0
Pressure of gases in alveolar air in mm. CO_2	39·2	37·1	39·8	41·2	48·2	67·7	39·3

From the known data of Shoji's blood, the line which represents the relation of CO_2 pressure to $1/K$ in the equation $F/100 = \frac{Kx^n}{1+Kx^n}$ at all pressures of CO_2 between 0 and 80 mm. is $1/K = 75{\cdot}7 \times p\,CO_2 + 600$.

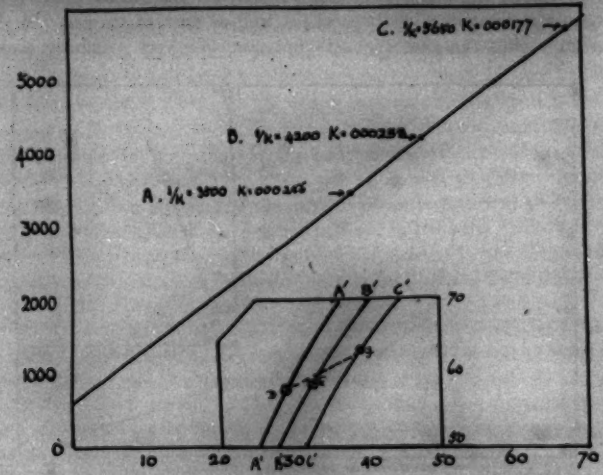

Fig. 3.

From this line set off on paper, as in Fig. 3, the values of $1/K$, and consequently those of $\log K$ for CO_2 pressures involved, are as follows:

	CO_2	$\log K$	Fig.
Exps. 1–4	39·3	$\bar{1}$·4564	A
5	48·2	$\bar{2}$·3766	B
6	67·7	$\bar{1}$·248	C

From these values of $\log K$ the corresponding dissociation curves AA', BB', CC' may be drawn, the portions involved being shown in the inset of Fig. 3. On these curves the observed oxygen readings are set off as follows.

Oxygen pressure	Curve	Percentage saturation of blood with oxygen
29	AA'	56·4
32	BB'	58·4
37	CC'	63·7

A line joining these points gives the percentage saturation of the blood, whatever be the CO_2 pressure in the alveolar air sample, between 38 and 68 mm., and which therefore embraces the CO_2 pressure of the mixed venous blood. If this line were quite horizontal, one could take any CO_2 pressure, draw the proper curve belonging to it and read off the percentage saturation of the mixed venous blood. As the line is not quite horizontal a slight correction must be introduced. The CO_2 pressure of the mixed venous blood was determined in Shoji's case by the method given by Christiansen, Douglas and Haldane(2). It was 49·5 mm. The proper curve for this pressure is scarcely distinguishable from *BB'*. The line *DE* crosses *BB'* at 58·4 p.c. saturation and is therefore a point 2 p.c. above the point *D* on the line *AA'* which was the uncorrected point as found by our method. The actual percentage saturation of Shoji's mixed venous blood may be obtained by adding 2 p.c. to the figure obtained by our method. A similar series of experiments on Barcroft's blood gives a correction of 3 p.c. to be added to the observed percentage saturation. Probably if the error is taken as 2·5 p.c. a result will be obtained which is commensurate with the general accuracy of the experiments.

Our reasons for believing that the experiments so far described with this correction, do give us a true measurement of the oxygen content of the mixed venous blood are strengthened, when we try to form a more concrete picture of what is happening in the lung, during the six-second interval in which the breath is held. During the whole of this period, mixed venous blood enters the pulmonary capillaries, charged with O_2 and CO_2, at certain definite partial pressures, and unless the latter are the same as the partial pressures of the corresponding gases in the alveoli at the beginning of the interval, diffusion exchange will take place until the partial pressure of O_2 and CO_2 in the alveoli are equal respectively to the partial pressure of O_2 and CO_2 in the mixed venous blood entering the capillary. Actually unless the composition of air breathed from the Douglas bag is carefully adjusted, six seconds will not be long enough to allow true equilibrium to be reached. For instance when pure N_2 is breathed from the bag, the alveolar sample as averaged from Exps. 1–4 of Table I only show $p\,O_2$ (partial pressure of oxygen) = 29 mm., and $p\,CO_2$ = 39·3 mm. If we could examine the condition of the blood at different points along a pulmonary capillary at the instant at which the alveolar sample was taken, we ought to find that $p\,O_2$ and $p\,CO_2$ gradually changed as we proceeded from the point where blood enters the capillary until we reached a certain point A_1, at which they equal 29

and 39·3 mm. respectively. At points of the capillary between A_1 and the point where the blood leaves, $p\,O_2$ and $p\,CO_2$ should be the same as at A_1. Similarly when N_2 containing 5 p.c. CO_2 is breathed, as in Exp. (5) of Table I, we ought, at the instant the six-second interval ends, to be able to find a point A_2 in the pulmonary capillary at which $p\,O_2$ of the blood = 32·2 mm., and $p\,CO_2$ = 48·2 mm. beyond which point $p\,O_2$ and $p\,CO_2$ are constant. A_2 lies nearer than A_1 to the point at which blood enters the capillary. Finally in Exp. (6), where N_2 containing a higher percentage of CO_2 was breathed, the corresponding point A_3 contains blood at higher $p\,O_2$ and $p\,CO_2$ than the mixed venous blood entering the capillary. The true $p\,O_2$ and $p\,CO_2$ of the mixed venous blood must therefore lie between the values corresponding to points A_2, A_3. If we plot $p\,CO_2$ against $p\,O_2$ for the three points A_1, A_2, A_3 as in Fig. 2 *B*, it is seen that A_1, A_2, A_3 lie not very far apart upon what is practically a straight line. It seems legitimate then to presume that the point *A*, which indicates $p\,O_2$ and $p\,CO_2$ of the mixed venous blood, also lies on the line A_1, A_2, A_3. The true $p\,CO_2$ for the mixed venous blood is measured with sufficient accuracy by the method of Christiansen, Douglas and Haldane(2), the point *A* on the line A_1, A_2, A_3 corresponding to this ordinate is observed, and the abscissa of *A* gives the true $p\,O_2$ for the mixed venous blood. Actually for the mixed venous blood we find that $p\,CO_2$ = 49·5 mm., $p\,O_2$ = 32·6 mm., and the percentage saturation calculated from Hill's equation = 58·5.

Comparison with Christiansen, Douglas and Haldane's CO_2 method (2).

An idea may be formed of the correctness of the method by a comparison between the oxygen lost from the blood, and that of the CO_2 which it gains. In Shoji's blood, taking the percentage saturation of the mixed venous blood with oxygen as 58·5 p.c. and that of the arterial blood as 95 p.c., the difference is 36·5. The oxygen capacity of Shoji's blood was 20·7 c.c. per 100 c.c. of blood. Therefore 100 c.c. of blood took in $\frac{36{\cdot}5 \times 20{\cdot}7}{100} = 7{\cdot}55$ c.c. We determined the CO_2 dissociation curve of Shoji's blood from which we obtained the following data. At a pressure of 39 mm. CO_2, his reduced blood contained 46·7 c.c. of CO_2 per 100 of reduced blood. According to the data given by Christiansen, Douglas and Haldane, the fully oxidised blood should contain 5·5 c.c. less CO_2 and arterial blood $\frac{5{\cdot}5 \times 95}{100}$ or 5·23 c.c. less than 46·7 c.c. The arterial

blood would therefore contain 41·5 c.c. By a similar calculation Shoji's mixed venous blood would contain $\left(51{\cdot}4-\frac{6\times 58{\cdot}5}{100}\right)=47{\cdot}9$ c.c. The difference between the CO_2 contents of the arterial and mixed venous blood would then be 6·4 c.c., which represents the CO_2 given out by 100 c.c. of blood. When this figure is divided by that for the O_2 taken in, namely, 7·5 c.c., a respiratory quotient of ·850 is obtained. It seems, then, that the methods described by Haldane and his colleagues for the CO_2 in the venous blood, and that described here for the O_2, give quite comparable results and that whichever is used as a basis for the calculation of the minute volume the result obtained would be nearly the same.

Comparison with the CO_2 method of Yandell Henderson.

Yandell Henderson(3) has described a method for the determination of the minute volume on the basis of a determination of the CO_2 in the mixed venous blood, which may be carried out in a few minutes. We made some determinations by Henderson's method and by our own with the following results:

Subject	O_2 given to 100 c.c. blood	CO_2 taken from 100 c.c. blood	Resp. quot. calculated	Resp. quot. observed	Minute volume (1) Present method	Minute volume (2) Henderson's
Redfield	4·7 c.c.	4·0 c.c.	·92	—	6·9 litres	6·65 litres
Barcroft	4·18	3·3	·8	·77		

Probably none of the above methods are very accurate, but for many purposes great accuracy is not required whilst a simple method is desirable. To obtain an ideal measurement of the blood flow there should be no change in the rate at which blood is driven round the body during the experiment. We have not accomplished this as satisfactorily as we could have wished. Clearly our initial respiration will tend to draw the blood which was in the veins to the heart. This, however, does not vitiate our result as the composition of the blood is that which it had when it left the capillaries. On the other hand, if the rate at which the blood passes through the systemic capillaries be altered, error will begin to appear when that blood begins to influence the composition of the air in the lung. In our experiments there is but one complete respiration of abnormal depth which will introduce a certain, but we believe, a trivial error. The final holding of the breath for six seconds does not affect the transit of any blood through the systemic capillaries which appears in the lung before the final expiration.

SUMMARY.

By the method of respiration described in the paper we believe that the coefficient of utilisation of oxygen from the blood may be measured within almost 10 p.c., from which the minute volume may be calculated to a corresponding degree of accuracy.

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VARIATIONS IN ALVEOLAR CARBON DIOXIDE PRESSURE IN RELATION TO MEALS; A FURTHER STUDY.

By E. C. DODDS and T. IZOD BENNETT, *Beit Memorial Fellow for Medical Research.*

(*From the Bland-Sutton Institute of Pathology, and the Department of Physiology, Middlesex Hospital.*)

In a previous number of this *Journal* (1) one of us (E. C. D.) drew attention to the changes in alveolar carbon dioxide pressure which follow the ingestion of a meal, showing that in the first half or three-quarters of an hour following the meal a rise of from 2 to 6 mm. occurs, with a subsequent fall below the original level and a return to that level during later stages in digestion. In a later paper (2), written in collaboration, we drew attention to the correlation which exists between the curves of gastric acidity and alveolar carbon dioxide pressure following meals, in normal individuals of varying type as regards their gastric secretion. The present series of experiments was designed to throw further light on the later portion of the curve of alveolar carbon dioxide pressure, *i.e.* that portion of the curve during which the pressure drops below the fasting level.

The effect of the direct introduction of substances into the duodenum.

Our subjects, who were healthy men working in our departments, were examined in the morning before any food had been taken. Ryle's modification of the Einhorn duodenal tube (3) was employed for the introduction of substances direct into the duodenum. The subject swallows the tube at about 9.30 a.m. After an hour and a half, or two hours, the withdrawal of a small quantity of alkaline bile-stained fluid is evidence that the tip of the tube has traversed the pylorus. Samples of alveolar air are now taken by the Haldane-Priestley method, and analysed with the Haldane apparatus; an inspiratory and an expiratory sample are taken at each reading, and the mean of the two readings expressed in terms of tension in mm. Hg. It having been ascertained that the subject's alveolar CO_2 pressure is at a constant level, substances are now introduced through the tube directly into the duodenum, by means of a "Record" syringe, and the variations in alveolar CO_2 pressure recorded. The food

substance used has, in the majority of cases, been oatmeal gruel prepared by the formula used in the preparation of gruel for fractional gastric analyses (4). In all cases the food thus introduced produced an immediate fall in alveolar CO_2 pressure with a subsequent return to the fasting level. In fact, the effect has been precisely similar to that seen in the latter half of the curve of alveolar CO_2 pressure following a meal taken in the ordinary way. A typical example of this experiment is seen in Fig. 1, details of which were as follows:

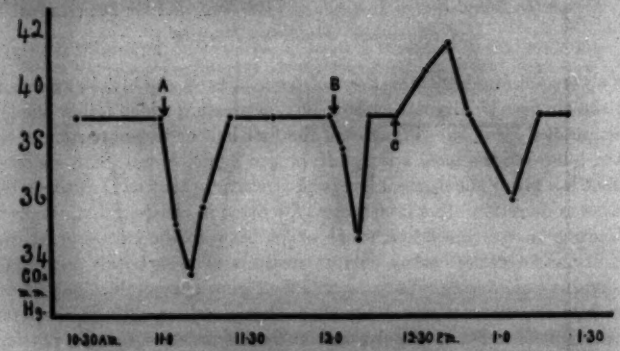

Fig. 1. Variations in alveolar CO_2 pressure produced by the direct introduction of substances into the duodenum.

In this and succeeding figures, abscissæ = time, ordinates = alveolar CO_2 pressure in mm. Hg.

Subject E. C. D. 7.30 a.m. Very light breakfast. 9.45. Duodenal tube swallowed. 10.20. Aspiration from tube yields a little alkaline bile-stained fluid. 11.2. 20 c.c. cold faintly alkaline gruel introduced into duodenum (A). 12.2 p.m. 20 c.c. faintly acid gruel introduced into duodenum (B). 1.26. Tube withdrawn 6 inches so that bulb now rests within stomach. 20 c.c. acid gruel introduced into stomach (C).

A series of experiments similar to the above has been performed, the result in all cases being to show (*a*) that the direct introduction of food substances into the duodenum leads to an immediate *fall* in alveolar CO_2 pressure with a subsequent return to the fasting level; (*b*) that variations in the acidity of the substance introduced do not produce appreciable difference in the response. In some cases a slightly greater response has been given by alkaline than by acid gruel; in other experiments the reverse has been true. Familiarity with the range of acidity of the gastric contents in man enables us to judge with fair precision what may be considered a safe limit in this respect. Food substances which are

definitely alkaline, on the other hand, are so rare that we have judged it unwise to introduce into the duodenum anything which is more than faintly alkaline to phenolphthalein. In Fig. 2 will be seen the result of the introduction of water of varying reaction into the duodenum; the response appears to be equally great in degree to that given by gruel.

In our previous paper(2) we produced evidence from a large number of human subjects to show that the increase in CO_2 pressure which follows the introduction of a meal into the stomach varies in degree

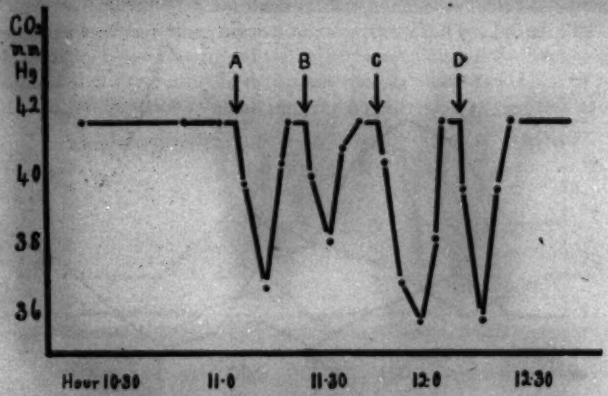

Fig. 2. Variations in alveolar CO_2 pressure produced by the direct introduction of water of varying reaction into the duodenum.

Subject J. W. B. 9.20 a.m. Subject, fasting, swallows duodenal tube. 10.20. Alkaline, bile-stained fluid aspirated. 11.5. 20 c.c. cold tap-water, acidified with HCl to a strength of 16 c.c. *N*/10, introduced into duodenum (A). 11.25. 20 c.c. water, just alkaline to phenolphthalein, introduced into duodenum (B). 11.46. 20 c.c. water, acidified with HCl to a strength of 40 c.c. *N*/10, introduced into duodenum (C). 12.10. 20 c.c. neutral tap-water introduced into duodenum (D).

directly with the amount of gastric HCl secreted by the particular individual; normal subjects, of average type as regards their gastric secretion, giving an alveolar CO_2 response of about 4 mm., those with marked hypersecretion as proved by repeated aspiration of abnormally large quantities of gastric juice, giving an alveolar CO_2 response sometimes as great as 12 mm., whilst those subjects, apparently normal in health, from whose stomachs HCl can never be obtained show no rise in alveolar CO_2 pressure whatever. These three types are reproduced in Fig. 3.

The rise in alveolar CO_2 pressure after a meal of fixed composition, gives an index of the amount of HCl secreted by the stomach of the individual concerned; moreover changes in the amount of HCl secreted, produced by varying the composition of the meal, are equally accurately indicated by alveolar CO_2 pressure observations; thus we have found that in normal human subjects of the usual type, substances such as meat extracts, known to excite an abundant gastric secretion, will produce a much greater rise in CO_2 pressure than is obtained by the administration of less active substances such as dextrose.

The immediate fall in alveolar CO_2 pressure shown in the present

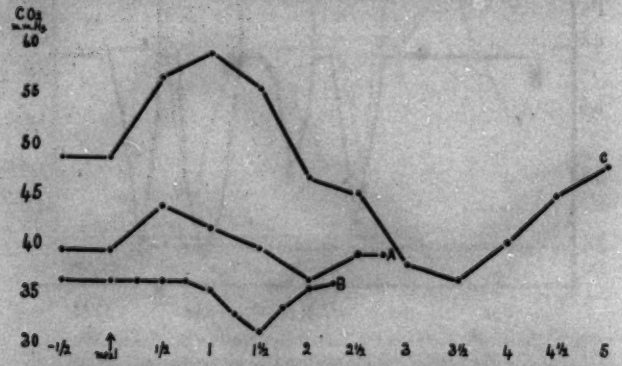

Fig. 3. Curves of alveolar CO_2 pressure following a fixed test-meal in healthy men proved to be of different types as regards their gastric secretion. A. From a subject of the usual average type. B. From a healthy subject with complete achlorhydria. C. From a healthy subject with marked gastric hypersecretion.

experiments to follow the direct introduction of food substances into the duodenum must, we believe, be the consequence of the secretion of alkali into that portion of the alimentary canal. Such alkaline secretion is at least threefold in origin, its sources being from (*a*) the duodenal mucosa, (*b*) the liver, (*c*) the pancreas.

The secretion of bile is believed to be continuous, if so it will have no periodic effect upon the reaction of the blood, and so will not influence the alveolar CO_2 pressure; of the remaining sources, it is indicated by the experimental work of Boldyreff(5) that pancreatic secretion is by far the more important. That such is indeed the case is supported by

an observation which we have been able to obtain from a patient suffering from chronic disease of the pancreas; for many years this subject has been unable to digest more than the smallest quantities of fat; the fæces contain unsplit fat and meat fibres in relative large amounts; the urinary diastase index is high; there is no jaundice nor sign of diabetes. An exploratory laparotomy some years ago revealed a fibrotic condition of the pancreas. In Fig. 4 will be seen the curve of alveolar CO_2 pressure following a test-meal in this subject as contrasted with the picture given by a normal individual of the more usual type. We hold that this observation lends further strong support to our belief that the fall of alveolar CO_2 pressure below its original fasting level, seen during the later stages of digestion of a test-meal, or in the experiments illustrated in this paper, is due to the secretion of alkali into the duodenum, and that this alkali

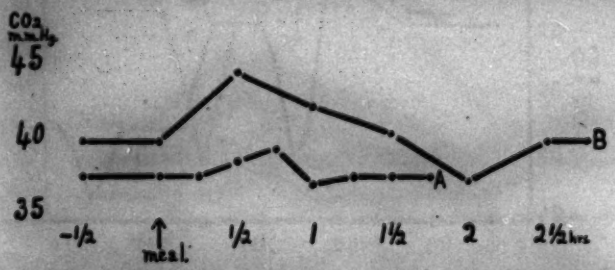

Fig. 4. Curves of alveolar CO_2 pressure following a test-meal in a subject with pancreatic deficiency (A). The curve from a healthy subject of the usual average type is reproduced for contrast (B).

is chiefly pancreatic in origin. If this belief be correct, then our observations, that the alveolar CO_2 response does not materially differ with substances of varying reaction, suggest that the formation of secretin by acid played no appreciable part in the production of pancreatic secretion in these cases. Further, since it is well established that perfect health can be maintained in cases of complete achlorhydria, we think that the formation of secretin by acid can only be regarded as an accessory, and relatively unimportant factor in pancreatic secretion.

The effect of the direct application of atropine to the stomach and duodenum.

By means of the duodenal tube either in the stomach or in the duodenum, atropine was applied locally, the response of the alveolar

CO_2 pressure to food substances introduced before and after the atropine application being recorded by alveolar air analysis. In Fig. 5 will be seen the effect of the local application of atropine to the gastric mucosa.

A further experiment illustrated in Fig. 6 shows the effect of direct application of atropine to the duodenal mucosa.

We interpret these two last experiments in the following manner:

In each case it is clear that the application of atropine has prevented the change in alveolar CO_2 pressure which normally follows the intro-

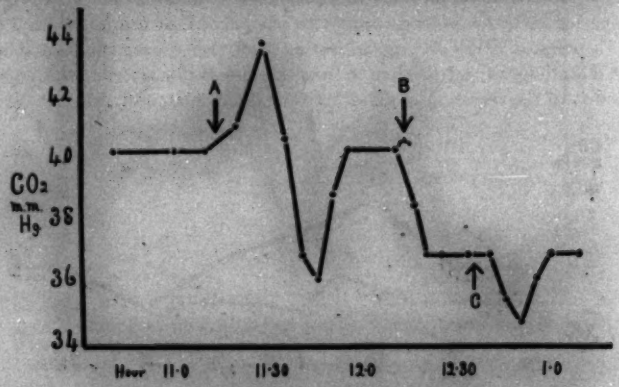

Fig. 5. Effect on alveolar CO_2 response to food, produced by local application of atropine to the stomach.

Subject T. I. B. 9.30 a.m. Tube swallowed into stomach. 10.4. Aspiration yields clear watery gastric juice in small quantities. 11.14. 20 c.c. neutral gruel introduced into stomach through tube (A). 12.13 to 12.16 p.m. Stomach gently washed with atropine solution consisting of $\frac{1}{12}$th grain atropine sulphate dissolved in 100 c.c. distilled water. It was introduced through tube 20 c.c. at a time, stomach being aspirated a moment later. In this way 100 c.c. atropine solution were introduced and about 105 c.c. subsequently withdrawn (B). 12.36. 20 c.c. neutral gruel introduced through tube into stomach (C).

duction of food into the viscus concerned; we believe this to be due to the inhibition of gastric secretions (in Exp. 3) and of the secretion of alkali into the duodenum (in Exp. 4).

More obscure is the interpretation of the change in "fasting level" of alveolar CO_2 pressure produced in these experiments. We believe that here the explanation is that continuous secretions have been inhibited by the local application. The continuous nature of salivary secretion is a matter of universal and everyday observation; Carlson has shown (6)

that in man, the gastric secretion, between meals, is of a similarly continuous nature; it would not therefore be remarkable if the alkaline secretion below the pylorus were of like nature, a slow continuous secretion giving way to a more rapid flow in response to the entry of food into the duodenum. Belief in such a conception is supported by these experiments, for it will explain how the local application of

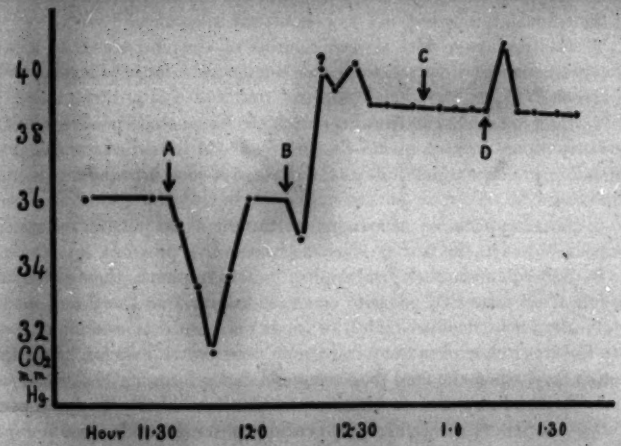

Fig. 6. Alveolar CO_2 response to direct injection of food into the duodenum before and after the local application of atropine to duodenal mucosa.

At the point marked ? on the chart the difference between the expiratory and inspiratory samples was too great to allow the mean to be fairly calculated.

Subject S. M. A. 9.45 a.m. The subject, fasting, swallows duodenal tube to its full extent. 11.10. Faintly alkaline bile-stained fluid aspirated. 11.35. 20 c.c. neutral gruel introduced direct into duodenum (A). 12.10. $\frac{1}{75}$th grain atropine sulphate in 20 c.c. water slowly introduced into duodenum. It is not possible to wash out the duodenum in a manner similar to the stomach. For this reason a rather stronger solution of atropine was employed and only about 3 c.c. of it were recovered by aspiration (B). 12.51. 20 c.c. gruel introduced direct into duodenum (C). 1.10. Tube withdrawn until bulb lies within stomach, 20 c.c. of gruel then introduced direct into stomach (D).

atropine to the gastric mucosa will not only inhibit the response to a meal, but will also, by stopping the continuous secretion of acid, cause an immediate decrease in the production of acid from the blood and a consequent fall in the alveolar CO_2 pressure; similarly we would explain the rise in the "fasting level" seen in Exp. 4 as being the result of the arrest of a continuous secretion of alkali into the duodenum.

Summary.

1. The direct introduction of oatmeal gruel into the duodenum, by injection through a duodenal tube, is followed by an immediate fall in alveolar CO_2 pressure.

2. The degree of this fall in pressure, which can be produced by water alone, appears to be independent of the reaction, acid or alkaline, of the substance injected.

3. Gastric lavage with a weak solution of atropine causes an arrest of continuous gastric secretion, and an immediate fall of the fasting level of alveolar CO_2 pressure.

4. Subsequent to gastric lavage with atropine no rise in alveolar CO_2 pressure occurs in response to the entry of food into the stomach, but the fall in pressure which follows the passage of food into the duodenum still occurs.

5. Local application of atropine to the duodenal mucosa causes an immediate rise in the fasting level of alveolar CO_2 pressure.

6. Subsequent to such local application of atropine to the duodenum, no fall of alveolar CO_2 pressure occurs in response to the direct introduction of food substances into the duodenum; but introduction of food into the stomach evokes the usual rise in pressure with no fall below the fasting level when the food passes beyond the pylorus.

7. In the light of data obtained from a case known to have pancreatic deficiency, the fall in CO_2 pressure observed under the circumstances described above is believed to be due to the secretion of alkali below the pylorus.

8. The respiratory centre allows changes of alveolar CO_2 pressure to occur whose tendency is to maintain the reaction of the blood at a constant level.

9. We conclude from our results that the formation of secretin by acid is not necessarily an important factor in producing pancreatic secretion.

The expenses of one of us (T. I. B.) incurred in connection with this work have been partly defrayed by a Government Grant made through the Royal Society. The respiratory observations form part of a report to the Medical Research Council.

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THE NATURE OF THE ISOMETRIC TWITCH.

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This paper is a consideration of experiments made upon a variety of questions arising in connection with the isometric muscle twitch.

(1) *The building up of the prolonged contraction.* A prolonged contraction is produced by the summation of the responses to a long succession of individual shocks. Tensions are additive, and if the isometric response to any later shock were the same as, or similar to, that to the first shock, the resulting contraction could be obtained simply by summing a series of shock curves of appropriate size, placed at regular intervals along the time axis. Conversely, if we knew both the curve of the isometric prolonged contraction, and the curve of the isometric twitch, it should be possible to analyse the former by means of the latter, in a manner similar to that employed in analysing curves of galvanometer deflection (1 p. 102), so determining how much of the prolonged contraction is produced by each one of the shocks causing it. We have attempted to carry out such an analysis, employing accurate photographic records, but the unreasonable results obtained have shown the analysis to be impossible, presumably because the response to a later shock was not similar to that to the first of the series. If the analysis be attempted it will be found that no succession of *similar* responses, corresponding to shocks up to, but not beyond, the end of the tetanus, is capable of being summed to give the observed curve of prolonged contraction, but that it is necessary to assume that the response to a later stimulus is different from and more drawn out than that to an earlier one. This conclusion is easily tested experimentally. If the ordinates of the curve of an isometric contraction, resulting from a maximal tetanus of 0·03 sec. (say), be subtracted at corresponding moments throughout its length from those of the curve resulting from a maximal tetanus of 0·06 sec., and the difference plotted against time, we obtain an expression for the response to the second 0·03 sec. of the 0·06 sec. tetanus. Similarly we may obtain the response to the third

[1] Working on behalf of the Medical Research Council.

0·03 sec. tetanus, by subtracting the ordinates of the curve of 0·06 sec. tetanus from those of 0·09 sec. and so on. Consequently we may construct a series of curves representing the responses to the first, second, third, fourth, etc., elements in a maximal tetanus of any desired duration. If this be done it will be found that these curves change progressively, the response to a later element in the stimulation being slower and more drawn out than that to an earlier element. The reason for this lengthening out of the response to successive elements of the stimulus must depend upon the actual physico-chemical nature of the events evoking contraction: it is possibly associated with the "mass action" effect of chemical bodies, produced in response to preceding stimuli, in slowing the forward velocity of the reactions occurring in contraction. Incidentally it explains the well known effect that a stimulus, of frequency just too low to cause complete summation of contractions, if persisted in, rapidly produces complete summation. In Fig. 1 are shown curves illustrating the change in the response to successive elements of a prolonged stimulus.

(2) *Temperature coefficients.* Observations have been made by many investigators of the temperature coefficients of various vital processes. In developing a theory of the internal mechanism of the muscle a knowledge of the effects of temperature on the various characteristics of the process is of value. The previous observations on muscle, however, have been made by various methods and under various conditions, and a mere bibliograph of them would be useless: it is necessary to standardise the methods and conditions and to employ the same tissue as is used in other investigations. We have carried out therefore a series of observations of the temperature coefficients of various characteristics of the isometric twitch of the frog's sartorius muscle. The muscle was stimulated directly by a maximal induction shock, recording its mechanical response photographically on a rapidly moving drum by means of a tension device of short period and high sensitivity. Typical records of the mechanical response are shown in Fig. 2 (*A*). The advantages of studying the isometric response are (*a*) that no complication can then result from a change of length, and (*b*) that errors due to the inertia of the recording device are reduced to a negligible minimum; it is possible therefore to study the rate of rise and the rate of decay of the response to stimulation in their simplest form. The advantages of employing the frog's sartorius muscle are (*a*) that nearly all our previous work on the heat-production has been done with it, its shape being singularly well adapted to such work, (*b*) that it is nearly of uniform cross-section, so that in an isometric

contraction one part of the muscle does not shorten at the expense of another, and (*c*) that being thin, oxygen can diffuse in rapidly, and so the condition of the muscle is maintained.

The initial extension of the muscle was comparatively small. The

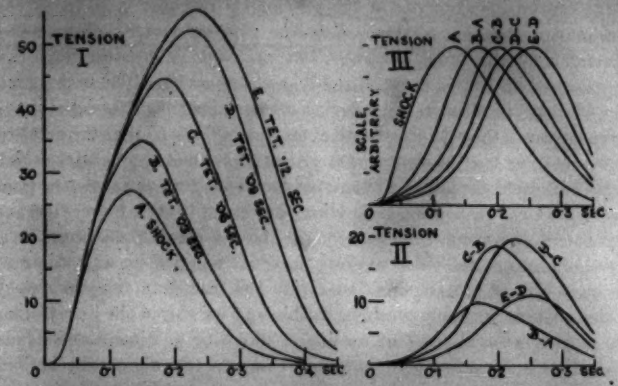

Fig. 1. Diagram showing response to successive elements in a prolonged maximal tetanus. Stimulation, by single-phase alternating current of 90 periods per sec., of sartorii of *Rana temp.* in Ringer's solution at 5° C. Isometric response recorded photographically: curves given contracted along the time-axis, for convenience of reproduction. Tensions in I and II expressed in units of 25 grms. wt. per sq. cm. of cross-section of muscle. Curves I reproduced directly from measurements of photographs representing the isometric response to (*A*) a maximal induction shock, (*B*) 0·03 sec., (*C*) 0·06 sec., (*D*) 0·09 sec., and (*E*) 0·12 sec. maximal tetanus. Curves II on the same scale represent differences between the heights of the Curves I. For example, (*B* – *A*) shows the ordinates of (*B*) less the corresponding ordinates of (*A*) and so represents the response to the rest of the first 0·03 sec. tetanus after the first shock. (*C* – *B*) similarly shows the ordinates of (*C*) less the corresponding ordinates of (*B*), and so represents the response to the second element of 0·03 sec. tetanus. (*D* – *C*) represents the response to the third element of 0·03 sec. tetanus, while (*E* – *D*) represents the response to the fourth element of 0·03 sec. tetanus. (*B* – *A*) actually begins at time O on the scale of curves I, (*C* – *B*) begins at time 0·03 sec., (*D* – *C*) at time 0·06 sec., and (*E* – *D*) at time 0·09 sec. on the same scale. In II each curve is drawn with its own zero at 0. It will be seen that successive equal elements of the stimulus produce very different responses, both in extent and character. Curves III (*A*), (*B* – *A*), etc., are the same as those similarly denoted in I and II, but drawn with a common maximum to show the characteristic lengthening out of the response. The development of the response to a later element of the stimulus occurs much more slowly than that to an earlier element. In the experiment shown in this figure the steady state, in which successive elements of the stimulus produce equal and similar responses, was reached in about 0·5 sec., in which case the maximum height of the difference curve for 0·03 sec. tetanus was 10 units.

character, as well as the size, of the response changes with initial extension (see p. 401 below), and it is necessary to avoid any possible complication from this cause.

(i) *Rate of development of the response.* The most satisfactory measure of the rate of development of the mechanical response is given by the maximum inclination of the record. The *point* of greatest slope is not determinable with much accuracy, but the *angle* of greatest slope can be measured off from a curve of the type shown in Fig. 2(*A*) with a high degree of precision, merely by laying a ruler along it. The slope of the curve measures the rate of increase of tension (dT/dt), so that the greatest slope measures the maximum rate of rise of tension, a quantity which seems well fitted for use as a characteristic of the rate of development of the mechanical response. It is clear, however, that a more vigorous response at the same temperature will show a greater maximum rate of rise of tension, and it is necessary, in order to allow for alterations in the mere size of the response, to divide the maximum rate of rise of tension by the maximum tension. In this way we obtain the "maximum rate of proportional rise" of tension, a quantity of dimensions minus one in time and similar to the velocity constant of a chemical reaction. It is this quantity whose temperature coefficient we have determined.

TABLE I.

			Exp. 1. June			
Temp. °C.	0·5	5	10	15	20	25
Max. rate of rise of tension / Max. tension	9·6	14·4	22·7	31·9	45·5	63·1
Temp. coeff. for 5° . .		1·50	1·57	1·41	1·43	1·40
			Exp. 2. June			
Temp. °C.	0·5	5	10	15	20	
Max. rate of rise of tension / Max. tension	10	15·1	23·7	38·2	61	
Temp. coeff. for 5° . .		1·51	1·57	1·61	1·57	
			Exp. 3. June			
Temp. °C.	0	5	10	15	20	
Max. rate of rise of tension / Max. tension	9·1	13·6	21·3	33·5	51	
Temp. coeff. for 5° . .		1·50	1·57	1·57	1·52	
			Exp. 4. June			
Temp. °C.	0	5	10	15	20	
Max. rate of rise of tension / Max. tension	8·9	12·9	23·0	33·3	49·0	
Temp. coeff. for 5°		1·45	1·78	1·45	1·48	

Summary of observed temperature coefficients for 5° C.

1·50, 1·57, 1·41, 1·43, 1·40 | 1·51, 1·57, 1·61, 1·57 | 1·50, 1·57, 1·57, 1·52 | 1·67, 1·72, 1·61, 1·25 | 1·45, 1·78, 1·45, 1·48 | 1·73, 1·48, 1·56, 1·31 | 1·53, 1·65, 1·54, 1·44 | 1·58 | 1·61 | 1·63 | 1·60 | 1·62 |

The numbers between any pair of vertical lines refer to the same experiment. The last five numbers were obtained by making an observation at one temperature, changing to a second temperature, returning to the first temperature again, and taking the mean of the first and third observations, so allowing for any progressive change in the muscle.

The temperature coefficient therefore of the rate of development of the mechanical response is practically constant over the range 0° C. to 20° C., and very reasonably constant for different muscles, having for experiments made in June an average of 1·55 for 5° C., or 2·40 for 10° C., and for experiments made in September—October an average of 1·60 for 5° C., or 2·56 for 10° C. The average for all experiments made is 2·50 for 10° C. The constancy of this value over the range 0° to 20° C., and its absolute size, are in keeping with the hypothesis that the rate of development of the response is determined by some chemical reaction, possibly by the action of lactic acid on the "sensitive" constituents of the muscle fibre.

The absolute value of the "maximum rate of proportional rise of tension" at a given temperature, say at 10° C., and at a given season, is a very constant quantity in the sartorius muscle. In the experiments made in June it had the following values at 10° C.: 22·7, 23·7, 21·3, 25·7, 23·0, 23·6, average 23·3: in the experiments made in September—October it had the values 20·3, 19·5, 19·2, 20·3, 15·7, 22·8, 18·7, average 19·5. The rate of response is rather less in September—October, than in June, as might be expected from the state of "training" of the animal. In this number we have an important physiological characteristic of the type of muscle and of the state of training or condition of the animal: by means of it it should be possible to compare one animal with another, or one muscle with another, or a muscle with itself under different conditions.

(ii) *Rate of disappearance of the response.* The most satisfactory measure of the rate of disappearance of the mechanical response is given similarly by the "maximum rate of proportional fall" of the tention. This quantity is obtained, as before, by measuring the maximum slope of the downward part of the curve, and dividing by the maximum tension. Table II gives a summary of the results. The quantities given are temperature coefficients for 5° C., and all the numbers within any pair of vertical lines refer to the same muscle over different temperature ranges.

TABLE II.

1·8, 1·8, 1·9 | 1·9, 1·6 | 2·7, 1·6, 1·6 | 2·1, 2·2, 1·9 | 1·6, 2·7, 1·7 | 2·1, 2·0, 2·3, 1·2 | 1·7, 2·4, 2·1, 1·5 | 1·8 | 1·6 | 1·7 | 1·8 |

Average, 1·90.

The numbers are not so regular as those given in Table I, and the average temperature coefficient found, viz. 1·9 for 5° or 3·6 for 10°, is higher than that of the rate of development of the response. The high temperature coefficient of the rate of disappearance of the response would seem to prove that relaxation is not due, as is sometimes suggested, simply to diffusion of lactic acid away from the sensitive surfaces of the fibril: it is difficult to see how diffusion could have a temperature coefficient of 3·6 per 10° C. We must look rather, as suggested elsewhere(4), to some chemical or physico-chemical reaction as the fundamental cause of relaxation. The difference between the temperature coefficient of the rate of development (2·5) of the mechanical response and that of its disappearance (3·6), suggests that different processes are involved, and that relaxation is not due simply to a reversal of the chemical processes involved in contraction[1].

The absolute value of the "maximum rate of proportional fall of tension" is a fairly constant quantity. In June at 10° C. it has the values: 4·6, 7·0, 10·2, 10·0, 9·2, 7·6, 8·3, average 8·1; and in September—October at 10° C. the values: 8·4, 9·6, 10·3, 10·5, 9·3, average 9·6. This quantity, averaging, say, 9·0 in all the experiments, is characteristic of the muscle and the animal at a given time and under given conditions, and could be used for comparative measurements of muscular activity.

It might have been expected that the record of the isometric twitch would be similar (in a geometrical sense) at different temperatures, or in other words that the record at 10° C. might be made to coincide with the record at 20° C. merely by changing the scales of times and tensions. This is not the case. The record at any one temperature is characteristic of that temperature, the effect of a rise of temperature being to quicken the disappearance more than the development of the mechanical response.

(iii) *Time occupied in the development of the response.* The records of the isometric response, as shown in Fig. 2 (*A*), give no means of determining an absolute value for the latent period. The curve leaves the base line gradually, and it is impossible to say when the contraction begins. By the use of a far greater magnification of the movement

[1] If the development of contraction were determined, for example, by the rate of diffusion of some body up to some surface in the fibre, then the rate of relaxation is not determined simply by the rate of diffusion of the same body away again.

Burdon Sanderson(5) found a real latent period of the order of 0·0025 sec. In order to obtain, from our records, some quantity characteristic of the time occupied in setting up the mechanical response we have adopted the following method. The line of greatest slope, which can be determined with great accuracy, was drawn in and the time interval θ between the shock and the point where the line of greatest slope cuts the base line, was measured up and compared at different temperatures. The following results were obtained, with nine different muscles, for the temperature coefficient, for 10° C., of $1/\theta$: 2·9, 2·5, 2·9, 2·85, 2·45, 2·7, 2·7, 2·4, 2·6, average 2·7. This high temperature coefficient again suggests that the time occupied in the development of the response is mainly occupied by chemical processes of some kind. The absolute values of this interval at 10° C. in the various experiments made were as follows: ·011, ·013, ·015, ·013, ·011, ·013, ·013, ·012, ·012, ·013, ·012, ·013, average ·0125 sec. The interval is very constant therefore (at 10° C. about 1/80 sec.) and may be used as a characteristic of the muscle for comparative purposes.

(iv) *Tension-time.* The area A of the tension-time curve, divided by T the maximum tension set up, viz. A/T, is clearly a measure of the time occupied in the twitch. A more rapid muscle will give a smaller quantity, a less rapid muscle a larger one. The effect of a rise of temperature is to diminish the duration of the contraction, and the temperature coefficient for 10° C. of T/A, *i.e.* of the inverse of the above quantity, had the following values with 11 different muscles, over various ranges: 3·6, 3·3, 2·8, 3·5, 2·9, 3·7, 2·2, 4·1, 3·9, 3·7, 3·7, 3·75, 3·7, 2·7, 3·7, 2·9, 4·0, 4·2. The average value is high, viz. 3·5.

The absolute value of A/T is an interesting quantity. It represents the time during which the maximum tension would have to be maintained in order to provide a tension curve of the same area as the actual contraction: in that sense it represents the "equivalent duration of the single twitch." It had the following values in 12 different muscles at 10° C.: 0·12, 0·14, 0·15, 0·15, 0·12, 0·15, 0·13, 0·14, 0·12, 0·13, 0·11, 0·14, average 0·13. In the above sense therefore the "equivalent duration of the single twitch" at 10° C. is 0·13 sec., and employing the temperature coefficient found above it would have the following values at other temperatures: 0° C. 0·45 sec.; 20° C. 0·037 sec.; 30° C. 0·011 sec.; and 37° C. (if the muscle did not go into rigor at this temperature) 0·0044 sec. In this quantity we have a good general characteristic of the time occupied in the complete cycle of contraction and relaxation, and one which might be valuable for purposes of comparison. The very small

"equivalent duration" at 37° C. is an expression of the inefficiency of the rapid voluntary muscle in *maintaining* a force at high temperatures.

(v) Tl/H. We have shown in another paper(4) that the quantity Tl/H is independent of temperature, H being heat, and T maximum tension developed in the isometric twitch of a muscle of length l. The experiments on which this conclusion was based were made on a variety of sartorius muscles, at different temperatures, the comparison of different muscles being made by expressing all the quantities involved in absolute units. This procedure was adopted to avoid the effects of fatigue, in experiments in which the determination of Tl/H in a twitch was only a small part. It was clearly desirable to substantiate it by experiments in which determinations of Tl/H were made, specially for the purpose, at different temperatures upon the same muscle, especially since Weizsäcker's experiments(9) on semi-membranosus muscles appear to show that in an isometric twitch T/H is increased some 40 p.c. by a rise of 10° C. We have devoted therefore several experiments to the elucidation of this question, controlling them carefully, as described below, to avoid any possible effects of fatigue. The tensions were recorded photographically, and the heat directly from the deflection of the galvanometer, two experiments being made with one thermopile, and two with another to avoid errors possibly arising from the use of one instrument only. The experiment was conducted as rapidly as possible, starting at 0° (or 20°), passing to 10°, and then to 20° (or 0°), and then repeating at 10° and 0° (or 20°), the mean values at any one temperature being taken to avoid the effects of fatigue. In three out of the four experiments the process was then repeated. The arrows indicate the order in which the experiment was conducted. Each number given is the mean of several observations agreeing well together.

TABLE III.

	Exp. 1. April. Female frog			Exp. 2. April. Male frog		
	0°	10°	20°	0°	10°	20°
Values of Tl/H	4·55 →	4·25 →	4·30	3·60 →	3·81 →	3·40
	4·08 ←	4·40 ←	—	3·13 ←	3·74 ←	—
Means	4·31	4·32	4·30	3·36	3·77	3·40

	Exp. 3. April. Female frog			Exp. 4. April. Male frog		
	0°	10°	20°	0°	10°	20°
Values of Tl/H	3·16 ←	3·83 ←	3·53	3·01 ←	3·31 ←	3·05
	— →	3·40 →	3·09	— →	3·34 →	2·69
Means	3·16	3·61	3·31	3·01	3·32	2·87

Exps. 2, 3 and 4 were continued and a second series of means in each case gave:

Exp.	0°	10°	20°
2	3·13	3·64	3·31
3	3·18	3·41	3·09
4	2·96	3·24	2·69

The average of all seven means at each temperature is as follows:

0°	10°	20°
3·30	3·61	3·28

From the table we see that there is apparently a tendency for Tl/H to increase as the temperature is raised from 0° to 10°, and to decrease again as the temperature is raised further from 10° to 20°. Over the whole range 0° to 20° the effect of temperature appears to be accurately *nil*: there is definitely a small rise between 0° and 10° and a small fall between 10° and 20°. What the origin of these small variations may be we cannot say: they appear to be genuine, but to be sufficiently small to be regarded as providing second order corrections to the statement that the primary effect of temperature upon Tl/H is *nil*. The constancy of Tl/H with temperature, in spite of the large changes in the time-course of the mechanical response and in T and H themselves, is strong evidence that the main effect of the chemical response (of which H is a measure) is the production of a state of elastic tension, measured by T.

(3) *The form of the curve of the isometric twitch.* The photographic method of recording the course of the isometric twitch gives so high a degree of accuracy that we hoped to be able to use it in finding a mathematical equation to the curve: such an equation—like that to the dissociation curve of hæmoglobin—might be of value in discussing the physico-chemical nature of the processes involved, and their changes with alterations of external conditions. We have tried a variety of equations, chiefly of the type occurring in mathematical physics (where a curve rising and falling like that of the muscle twitch is fairly common). None of these in general has given a satisfactory fit; occasional success has been achieved with the equation $y = t^n e^{-\lambda t}$, where y is the tension at any moment t, and λ and n are constants, but this equation often gives only poor accord with the actual curve. There may not of course be any simple equation to the curve, but it is possible that the difficulty in finding one may lie in a disturbance to be described immediately, which obviously occurs in many instances, and possibly occurs in all. The disturbance consists of a "hump" on the tension curve, as shown in Fig. 2(*B*). We have been unable to ascertain the cause of this disturbance, but have discovered various factors connected with it. Until its origin

is clearer it is obviously impossible to assume that it is at any time absent from a record, and useless therefore to attempt to fit a simple equation to the curve.

In Fig. 2 (*A*) are shown two photographic records at 0° C., apparently smooth and undisturbed. The variation between them is striking. In Fig. 2 (*b*) are shown four records of twitches and one record of a short tetanus, in which noticeable "humps" occur. It was thought at first that this might somehow be due to the presence of nerves, or nerve endings, in the stimulated muscle, a shock both to the nerve and to the muscle fibre causing a double "summated" contraction: the curves are not unlike those produced in response to a tetanus. That the disturbance

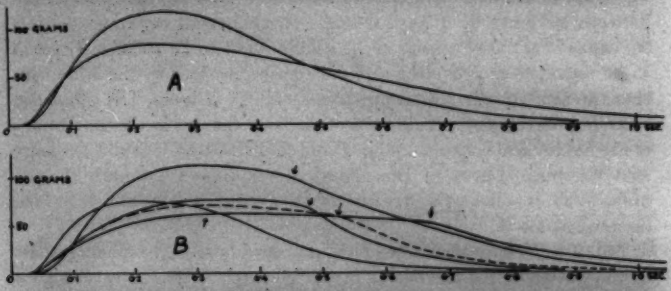

Fig. 2. Isometric twitches of sartorius of *Rana temp.* at 0° C., reproduced directly from photographic record. (*A*) Smooth normal records of twitches of two different muscles. (*B*) Four twitches from different muscles, and one short tetanus (0·03 sec. tetanus, shown as a broken line), giving an obvious "hump" at the point marked by the arrow.

is not due, however, to the presence of nerves and nerve endings in the muscle is shown by the fact that it occurs just as obviously after treating the muscle for 1 hour with 0·25 p.c. curare. It is more obvious at low temperatures than at high: it usually occurs, sometimes violently, at 0° C., but it is often distinct at higher temperatures. Its position on the curve at 0° C. varies from 0·3 to 0·7 sec. after the shock, for no apparent reason. It is greater at small initial extensions than at large: it occurs both in oxygen and in Ringer's solution: exercise appears to diminish it: it is not abolished by using non-polarisable electrodes[1]: it occurs

[1] We thought that it might be due to a second shock provided by the back current caused by the polarisation produced by the first shock. That this is not so is shown by the fact that it occurs equally plainly when the electrodes are of silver, well coated with AgCl, or of the porous pot type as made by the Harvard Apparatus Company.

equally in maximal and in sub-maximal twitches. The only clue we have found as to its origin lies in the nature of the contacts at the electrodes. The muscles employed were the sartorii of *Rana temp.*, joined together at their pelvic ends, and tied by silk threads at their distal ends to the tension device. Whether in oxygen, or in Ringer's solution, one electrode was in contact with the bone and muscle at the pelvic end, and the other electrode consisted of a loop of stout platinum wire wound round the distal ends together, about 4 mm. from the tendons. A poor contact with the electrode at the pelvic end appeared to have no effect in producing a "hump," but a poor contact at the distal end led at once to a fairly obvious one. In Ringer's solution the removal of the electrode from actual contact with the muscle at its distal end appeared to be a certain prelude to a "hump," which a good contact would abolish or reduce. The time at which the "hump" occurs, viz. (at 0° C.) 0·3 to 0·7 sec. after the shock, would appear to preclude the possibility of its being due to the slow passage of the contraction wave along the muscle, for this wave could scarcely travel as little as 3 cm. in (say) half a second, even at 0° C. There would seem also to be no possibility of the "hump" having a purely mechanical origin (due *e.g.* to the muscle slipping suddenly when a certain tension is reached) for the muscles were free in air or Ringer's solution, and held firmly at each end. We can only suppose that the phenomenon has a physiological origin, being due perhaps to the superimposed contractions of two separate elements in the muscle or the muscle fibre.

(4) *The effect of initial extension on the isometric twitch.* In recent papers Doi(6, 7, 8) has shown the effect of initial extension of the muscle upon (*a*) the maximum force developed, (*b*) the maximum work done and (*c*) the heat produced, in a maximal isometric twitch. In each case the effect of increasing extension was the same, viz. an increase of the quantity observed up to a maximum value, and a subsequent fall. We have been able to confirm Doi's experiments on the effects of extension on the heat-production, and on the maximum force developed in a twitch, and find (as he did) that these usually attain a maximum at a relative length of 1·4 or 1·5. In addition we have determined the effects of extension on a variety of other factors in the twitch. The results are shown in tabular form below.

TABLE IV. *The effects of extension on the muscle twitch.*

(1) Heat production, H: maximum for relative length 1·4 to 1·5: Effect of temperature diminished considerably by extension.
(2) Tension-time, $\int T\,dt$: maximum for relative length 1·4 to 1·5: Effect of temperature diminished considerably by extension.

(3) Tension-time/Heat production: maximum for relative length 1·4 to 1·5: Effect of temperature unchanged by extension.
(4) Tension-time/Maximum tension: increases continually and considerably with extension. Effect of temperature unchanged by extension.
(5) H/Maximum tension: increases continually and considerably with extension. Effect of temperature unchanged by extension.
(6) Time of development of response (time between the shock and the point where the line of maximum upward slope cuts the base line): independent of extensions: temperature coefficient unaffected by extension.

It may be of interest also to record that the calibration constant of the thermopile (heat in gram.-cm. per mm. deflection of the galvanometer) is unaffected by the extension of the muscle. This had not been expected, and the fact that it is so is fortunate, and will be of value in experiments in which the muscle is allowed to shorten on the thermopile. The following results are typical.

Relative length . . .	1·29	1·44	1·55	1·55	1·44	1·29
Gram.-cm. per mm. deflection	0·237	0·234	0·230	0·234	0·234	0·230

Incidentally this experiment shows the great consistency of the method of calibration: the six observations recorded extended well over an hour.

The most striking change, however, produced by an extension of the muscle, is in the character of the response. In Fig. 3 (A) are given records of isometric twitches, at 10° C., in which the muscle was subjected to increasing extensions. In order to avoid any possible effect of fatigue (or of mechanical injury by the extension) the series was repeated in the reverse order, maximal shocks being given in succession at relative lengths: 1·19, 1·29, 1·40, 1·49, 1·54, 1·59, 1·54, 1·49, 1·40, 1·29, 1·19. The ordinates at intervals of 0·02 sec. were then measured up from the photographic records, and the means were taken for each pair of records at the same extension. In this way mean curves were constructed, characteristic of each extension, and it is these curves which are shown in Fig. 3 (A). In order to emphasise the change in the character of the record, as well as in its height, similar curves are shown in Fig. 3 (B) reduced to a constant maximum. From the latter figure we see that an increasing extension leads to a progressive fall in the rate of *relaxation*. This effect is not a small one: indeed an increase in relative length from 1·19 to 1·59 decreases almost to one third the rate of relaxation. It is difficult to picture any physical basis for this effect. If relaxation be due, as is probable, to the removal of lactic acid by some physico-chemical combination from the surfaces or spaces where it works, then the increase in length or tension must somehow delay the process of removal. Whatever be the true theoretical explanation of the effect, it has a certain physiological and practical significance; for example the lengthening out of the contraction by an extension of the muscle will secure a complete summation of contractions with a smaller frequency of stimulation and so cause a prolonged contraction to be maintained with a greater "efficiency" [see (4), p. 148]. It would be of interest to investigate whether a

similar effect of extension occurs in cardiac muscle, where it might be of great concern to the animal.

(5) *Variation of stimulus, and the "all or none" principle.* If a pair of sartorius muscles be made to carry out a series of isometric twitches, in response to a succession of break induction-shocks of increasing

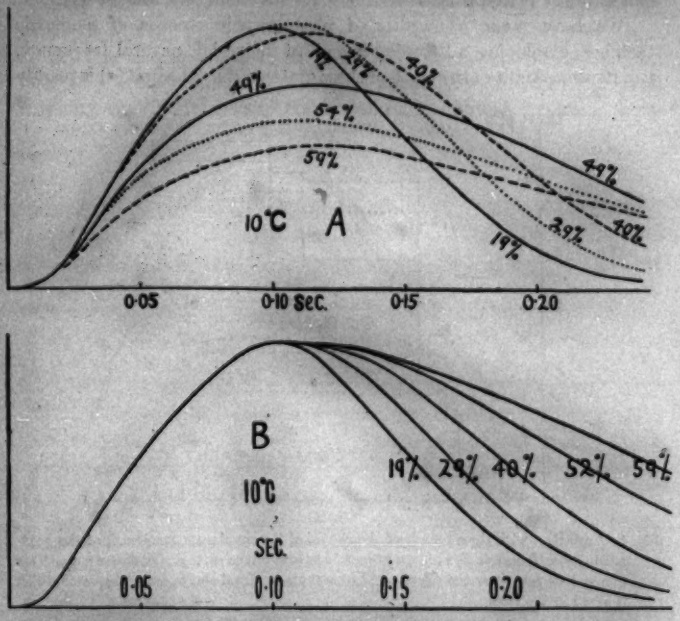

Fig. 3. Isometric twitches of sartorius of *Rana temp.*, at 10° C., at various extensions expressed in percentages of the resting length. Curves (*A*) represent actual means of two series taken respectively with increasing and decreasing extensions, to eliminate possible effects of injury or fatigue. Curves (*B*) have been reduced to a constant maximum, to show the effect of extension on the character of the twitch.

strength (varied either by increasing the current in the primary, or by shifting the secondary coil), the mechanical response will be found to vary in the following manner:

(*a*) *T*, the maximum tension developed, increases *in steps* as the shock is increased, as shown in Fig. 4, reaching a perfectly definite

maximum value beyond which no increase in the shock has any effect, either on the height or on the shape of the curve;

(*b*) The *form* (as distinguished from the height) of the tension-time curve changes continuously in a regular manner, as shown in Fig. 5: the curve rises at almost exactly the same relative rate in all cases, while in a stronger twitch it falls relatively quicker than in a weaker one.

We have made a number of photographic records of isometric twitches, employing a "tension-lever" of very high natural frequency, and have no doubt of the truth of these statements. The first (*a*) is readily

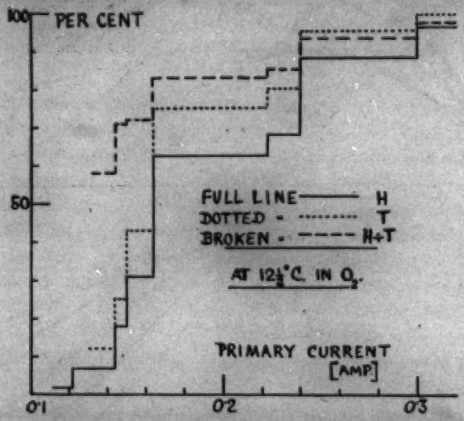

Fig. 4. Variation, with the strength of the shock, of the maximum tension T, of the heat-production H, and of H/T, in an isometric twitch of the sartorius of *Rana temp.* This figure does not show the effect on H of a supramaximal shock, an example of which is given in the text.

intelligible in view of Keith Lucas'(2) proof that the sartorius muscle contains several different excitable tissues, which come into play at different levels of the stimulus: and our experiments have entirely confirmed Mines'(3) observations of the same phenomenon. Moreover, it can easily be shown that after treatment with curare the steps are abolished, the maximum tension increasing continuously to a maximum as the stimulus is increased, owing to the direct excitation of a continuously increasing number of muscle fibres. The second phenomenon (*b*) is not so easy to explain. If the effect of increasing the shock be merely to increase the number of fibres reacting to it, the stronger twitch should

be merely a replica of the weaker twitch on a larger scale. We must conclude therefore, either that the sartorius is not composed of similar parallel fibres, or that the individual fibre responds in a different manner to a stronger shock.

If now the muscles employed be placed upon a thermopile, and the "initial" heat-production recorded as well, the thermal response will be found to vary in the following manner as the shock is increased:

(*a*) The heat-production H increases, exactly like the maximum tension T, in a series of steps (which can similarly be abolished by curare) reaching a perfectly definite maximum value: beyond this value, how-

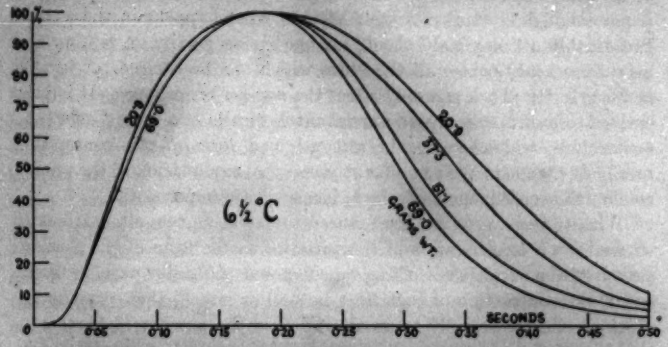

Fig. 5. Examples of maximal and submaximal isometric twitches of sartorius of *Rana temp.* at 6½° C. The curves are plotted with the same maximum to show the differences in the form of the twitch, each curve being marked with a number denoting the maximum tension (in grms. wt.) developed in it. Note that the weaker twitch invariably shows the slower relaxation, while the rate of development of the response is practically the same in all.

ever, a further increase in the shock causes a *diminution* in the heat, which diminution may be anywhere from zero to 15 p.c. of the maximum value.

(*b*) The value of H/T increases, as the shock and T increase, by an amount varying from 11 p.c. to 35 p.c., and averaging 26 p.c. in our experiments. When, however, the shock is increased beyond its maximal value, H tends to decrease and T to remain constant, so that H/T tends to decrease by an amount up to 15 p.c. of its maximum value.

Of these phenomena also we have made a number of observations, on muscles in good condition in oxygen, and have no doubt of their

existence. With regard to (*a*), the diminution in the heat-production resulting from the increase in the shock occurred to an obvious degree in only about one-third of the muscles investigated: in the remaining two-thirds it was small or negligible. We are unable to say why the phenomenon should occur in some cases and not in others, but the fact that it can occur at all is sufficient for our argument. It is obvious that if increasing the shock increases in steps the number of fibres responding to it, the heat-production should increase in steps also. The fact, however, that an increase of the shock, beyond the value required to give maximum heat and maximum tension, tends to result in an appreciable diminution of the heat, while leaving the mechanical response absolutely unchanged, is not intelligible on any existing view of what happens in the muscle. Presumably a "maximal" shock, *i.e.* one giving maximum tension and maximum heat, excites all the fibres which can be excited. A further increase in the shock can only affect the *manner* of response. It is very curious to find that a supramaximal shock results in a more "efficient" contraction, while leaving the strength and form of the contraction totally unchanged. We can offer at present no explanation of the phenomenon, the consequences of which, however, are important.

With regard to (*b*), we are reduced to the same two alternatives as we were by a consideration of the variation in the form of the tension-curve. If the sartorius of a frog be composed of similar parallel fibres, and if the contraction of each fibre be "all or none," then varying the shock should lead simply to an alteration in the number of fibres contracting, H/T remaining unchanged. H/T, however, has been shown to carry out a complicated change, increasing considerably to a maximum and then tending to decrease again to a minimum, as the shock is continually increased. From this, in conjunction with (*a*), we can only conclude that it is possible, by varying the shock, to vary—at any rate within certain limits—the heat-production, if not also the rise of tension and the form of the response, associated with the twitch of the *individual* muscle fibre. Varying merely the number of fibres reacting, clearly cannot change H without changing T. To this extent therefore the "all or none" principle fails: the single fibre has been shown to respond in a different manner to a different single shock.

We give below one experiment, from among many, illustrating the phenomena in question.

(6) *The recovery*[1] *process of contractile tissues.* In recent papers on

[1] It should be noted that the recovery processes referred to here have nothing to do with the oxidative recovery process lasting for some minutes after exeitation: they imply

Frog's sartorius muscles in O_2 *at* 10° C. *July.*

1. Altering position of secondary coil: primary current 0·8 amp.

Position of coil	6	6½	7	8	9	10	11	12	13
$H \longrightarrow$	190	190	195	200	206	209	209	206	175
$H \longleftarrow$	189	189	193	192	198	211	208	206	—
Mean H	189·5	189·5	194	196	202	210	208·5	206	175

2. Altering strength of primary current: coil at 6 (continuation of above).

Current in primary; amps.	0·8	0·64	0·53	0·45	0·40	0·36	0·29
$H \longrightarrow$	189	190	192	196	200	202	209
Current in primary; amps.	0·25	0·20	0·16	0·13	0·06	0·055	0·045
$H \longrightarrow$	209	211	212	213	211	191	131

Note. The observations were made in the order shown by the arrows.

"The recovery process of excitable tissues," Adrian(10, 11) has discussed the gradual return of normal excitability following a shock. He has shown in nerve that after a shock the *excitability*—at first zero during the absolute refractory period—gradually increases, and that in a medium on the acid side of neutrality the excitability passes through a supernormal phase before settling down finally to its initial value. He has also shown that the *contractile power* of a heart passes through the same process, showing the same supernormal phase in a slightly acid medium. In skeletal muscle nothing of the kind has yet been established. The so-called "staircase" effect in skeletal muscle is due, as Fröhlich(12) and Adrian(10) have shown, simply to the effect of fatigue in lengthening the contraction, and to the use of a recording device possessing too much inertia. Clearly if the recovery process established for cardiac muscle occurs, in the case of skeletal muscle, during the contraction itself it is not easy, merely by recording the mechanical response, to investigate it. As a matter of fact, as will be shown below, it is possible by using rigidly isometric contractions to do so, but a more convincing method is to measure the response of the muscle by measuring its heat-production when stimulated, (*a*) by one shock, and (*b*) by two shocks separated by any desired interval. It is necessary in these experiments to work only with muscles which do not show, to any appreciable degree, the phenomenon described above, viz., the diminution in the heat-production occurring in response to a supramaximal shock. If a muscle be subjected to a single maximal shock it gives out a certain amount of heat, say 100. If the same muscle be now subjected to a pair of shocks, the first being maximal and the second well exceeding maximal (to ensure its maximal nature), the two shocks being separated by any desired interval, then

merely the rapid restoration of the readiness to carry out the initial breakdown processes of contraction.

the pair of shocks will produce more heat, say in any given instance 131, so that the second of the two has produced heat 31, and the energy-liberating power of the muscle in response to a maximal shock has recovered to 31 p.c. of its original value. Experiments made in this way have shown that a sartorius muscle, directly stimulated, shows precisely the same recovery of contractile power (in this case more precisely energy-liberating power) as the heart, but with very different time-relations, and moreover that under certain ill-defined conditions the power of the

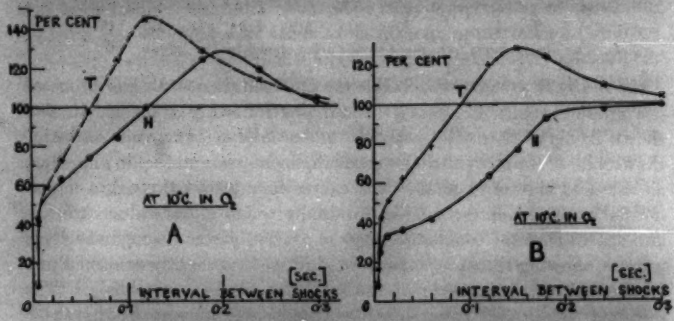

Fig. 6. To illustrate recovery of skeletal muscle following a shock. A single maximal shock is given, causing an isometric twitch with an evolution of heat H, or a development of tension T, represented by 100 in the figure. Two maximal shocks are then given, at any desired interval, and the heat, or tension, produced by the *second* shock determined as described in the text, and plotted against the value of the interval. The contractile power, represented by the T-curve, starting from zero during the absolute refractory period, undergoes a process of recovery, always passing through a supernormal phase before settling down to its original value. The energy-liberating power, represented by the H-curve, shows the same progressive changes, sometimes passing through the supernormal phase (as in A), sometimes reaching its original value from below (as in B). The H and T curves are widely different, the value of T/H always passing through a prolonged supernormal phase before settling down to its original value.

muscle to respond to the second shock passes through the same supernormal phase. Typical results are shown in the curves marked H in Fig. 6 (A) and (B). It will be noticed that, during what is presumably the absolute refractory period, the second shock following the first at a very short interval produces no heat at all, while as the interval is increased the heat production due to the second shock increases to its full value, in some cases as in (A) passing through a supernormal phase in which the heat is some 30 p.c. greater than that due to a single

maximal shock. It will be noticed from the time-relations that the recovery process occurs largely during the period occupied by the active mechanical response. (Compare, for example, Fig. 3 at the same temperature.) It can be shown also that it goes on much more slowly at a lower temperature, the effect of temperature being somewhat irregular. There are two separate factors involved in this process of recovery: firstly there is the return to normal excitability, secondly there is the restoration of the "ready store" of energy (see Hartree and Hill(4)) following the expenditure evoked by the first shock. It was shown in our previous paper(4), and by Doi(8), that the expenditure of energy is greater in a single twitch at a lower temperature, and that the restoration of the "ready store" of energy is considerably slower at the lower temperature. Thus at the lower temperature the more depleted store is replenished more slowly, while the excitability is restored more slowly, and the combination of these several independent factors leads to an excessive and somewhat irregular effect of temperature on the recovery process.

The same phenomena can be exhibited by considering the mechanical response and are shown by the curves marked *T* in Fig. 6. If the isometric tension-curve be recorded as before, on a rapidly moving drum, (*a*) for a single maximal shock, and (*b*) for two maximal shocks at any desired interval, then by subtracting the ordinates of the first curve from those of the second, and plotting the difference, it is possible to construct the mechanical response produced by the second shock. Fig. 7 gives examples of such a process. If the maximum tension produced in the response to the second shock be calculated in this way, and plotted against the interval between the shocks, we find a relation of the same type as is shown by the heat-production, the only difference being that the contractile power shows an even greater supernormal phase than does the heat-production, thus demonstrating that not only the power, but the "efficiency" of the muscle is increased during this phase. An example of the relation between the maximum tension developed in the response to the second shock, and the interval between the shocks, is given in Fig. 6, for comparison with a simultaneous set of observations relating the heat produced by the second shock to the same interval. It is seen that the supernormal phase occurs earlier with the tension than with the heat-production, the curves being by no means similar.

The phase of supernormal heat-production does not always occur. In some cases the heat-production increases continually to its maximum, as the interval is increased. We have made a large number of experiments

in an attempt to determine the factors responsible for the presence, or absence, of this supernormal heat-production, but with no success. In view of Adrian's(10) results on the effects of hydrogen-ion concentration in determining the presence, or absence, of a phase of supernormal excitability, it was natural to proceed to an investigation of its effects in our case also. The following experiments show that the C_H is not

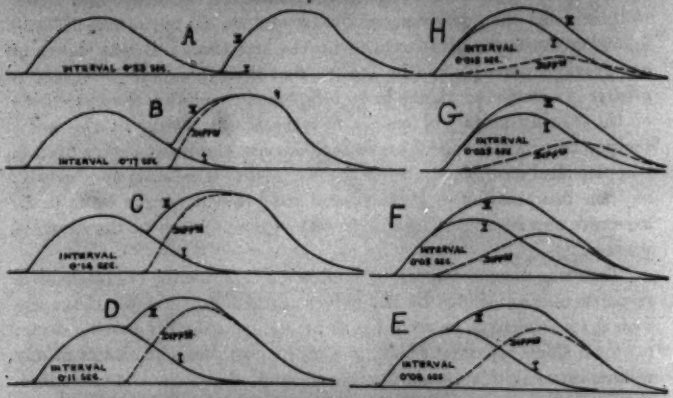

Fig. 7. To illustrate return of contractile power following a shock. In every case the curve marked I represents the isometric twitch of the sartorius of *Rana temp.* in O_2 at 10° C. in response to a single maximal induction shock. The curve marked II represents the later part of the response to a pair of maximal induction shocks, at the interval noted, the earlier part of the response being curve I up to the point where it joins II. The broken line represents the difference between the response to the two shocks and the response to the single shock, *i.e.* represents the response to the second shock when given at the interval noted. In *A* the second shock is given just as the first response has passed off; the difference coincides with II and the response to the second shock is slightly supernormal. In *B*, *C* and *D*, with decreasing interval, the response to the second shock is very distinctly supernormal: in *E*, with smaller interval, it is only just supernormal again, while in *F*, *G*, and *H*, with still smaller intervals, it is increasingly subnormal. The maximum height of the difference curve, plotted against the interval, provides a relation similar to that marked *T* in Fig. 6.

the determining factor for the presence or absence of a supernormal heat-production. In each experiment the muscle was immersed, on the thermopile, in a buffered Ringer's solution of known p_H (as used by Adrian(10) and Mines(13)) and a curve made relating the heat-production caused by the second shock (expressed as a percentage of that caused by the first) to the interval between the shocks. The maximum value

of the heat-production of the second shock is denoted by "max. H.P.," and the corresponding interval between the shocks is denoted by "interval." All experiments were at 10° C. In some cases experiments were made in O_2 (or N_2) after not less than ¾ hour immersion in Ringer's fluid: these are denoted by O_2 (or N_2) in the table: observations made on muscles actually in Ringer's solution at the time are denoted by *R*.

Exp. No.	1		2	3		4			
p_H	7·4	7·4	7·4	5·5	5·5	8·5	8·5	7·4	7·4
Medium	O_2	O_2	O_2	R	O_2	R	O_2	R	O_2
Max. H.P.	123	103*	127	100	114*	107	110*	100*	107*
Interval	·18	·15	·18	—	·21	·18	—	—	·18

Exp. No.	5		6			7		8	
p_H	7·4	5·5	8·5	8·5	8·5	5·5	5·5	7·4	8·5
Medium	R	R	R	O_2	O_2	R	O_2	O_2	O_2
Max. H.P.	132	112*	111	117*	117*	105	112*	110	100*
Interval	·24	·24	·21	·21	—	·15	—	·13	—

Exp. No.	9		10		11		
p_H	7·4	7·4	7·4	7·4	7·4	7·4	
Medium	O_2	N_2	O_2	N_2	O_2	O_2	
Max. H.P.	100	128	120*	100	100*	114*	
Interval	—	·18	—	—	—	·27	

* Later, after an interval of at least an hour.

In contradistinction to the phase of supernormal heat-production, which does not always occur, the phase of supernormal mechanical response appears to occur under all conditions. The second shock, after a suitable interval, invariably gives a greater maximum tension than the first shock, and the supernormal phase persists (at 10° C.) so long that the tension curves may be entirely separated, and yet the second be higher than the first. This is clearly seen in Fig. 7.

Of the mechanism of this supernormal phase we are completely ignorant. It is not apparently affected by the C_H of the fluid, by the presence or absence of O_2, or by continued exercise. Of one thing, however, we may be reasonably certain, viz. that it represents a true "staircase" effect in skeletal muscle, exactly analogous to that occurring in cardiac muscle but with different time-relations. The so-called "staircase" effect in skeletal muscle is an artefact, due to the lengthening out of the contraction of a fatigued muscle, and to inadequate recording arrangements: the same inadequate recording arrangements have delayed the discovery of the real "staircase" effect described here. It is seen also, in Fig. 7, that the mechanical response to the second shock, as determined in the manner described above, is quite different in character (as well as in size) from the normal response; this confirms the conclusion that the summed contraction cannot be considered as the

resultant of a number of *similar* elements of contraction evoked by successive elements of the stimulus.

A further point of interest arises in connection with the curves of Fig. 6: the T-curve lies above the H-curve, in A for a long time and in B for all the time. In other words the "efficiency" of the contraction, as measured by the ratio T/H, can be considerably increased by a previous shock at a suitable interval. If the same rise of efficiency occurs in the analogous case of cardiac muscle it may be a matter of importance in the economy of the heart.

(7) *The effects of curare.* Curare has a variety of effects on the contraction of the frog's sartorius, stimulated directly, some of which can be explained by the specific action of curare in paralysing the nerve endings; two, however, can not. Of these two, the most remarkable perhaps is that the application of 0·25 p.c. curare regularly increases the output of heat in the twitch by some 25 p.c., increasing the maximum tension by only 8 p.c. and somewhat prolonging the contraction. Whether the effect of curare be to prolong the rise of permeability following the stimulus, or whether it be to increase the store of energy ready to be discharged during that rise, (see (4)) we cannot say: veratrin almost certainly acts on the former process, and it would appear possible that curare acts, to a very minor degree, in the same way as veratrin. The second effect of curare is to maintain the life and condition of the muscle far longer than is usual in a muscle resting on a thermopile. It has always been noticed, at some times more than others, that muscles resting on a thermopile tend to deteriorate and die far more rapidly than a normal surviving muscle in oxygen should. We have been led to attribute this to some unknown harmful constituent of the shellac used for insulating the thermopile. The effect of curare is apparently to prevent this deleterious influence of the shellac, and to cause the muscle to continue in good condition for long periods, as it would if suspended in oxygen.

Summary.

1. A prolonged contraction cannot be considered as the resultant of a number of *similar* units occurring in succession. The response to a later element of stimulus is different in character from that to an earlier element. Experiments are given showing the response to successive elements of a prolonged stimulus.

2. The effects are described of a change of temperature upon various characteristics of an isometric twitch: T/H is independent of temperature.

3. The form of the isometric twitch is discussed, and the occasional occurrence of a "hump" on the curve is described.

4. Extension of the muscle results in a considerable prolongation of the twitch, owing to a slowing of relaxation.

5. As the strength of a shock is increased, the heat-production increases to a maximum in a series of steps, and then tends to fall to a value not more than 15 p.c. lower than the maximum. The tension also rises to a maximum in steps, but then remains constant. As the shock is increased, H/T increases to a maximum and then falls off, and the curve of the response changes its shape. From these facts it is concluded that, at any rate within limits, the "all or none" principle fails and that the individual fibre can respond in a different manner to a different shock.

6. The recovery process of the contractile change is considered. If two maximal shocks be sent into a muscle in succession, at any desired interval, the thermal or the mechanical response of the muscle to the second shock can be determined. It is found that under all conditions there is a phase of supernormal mechanical response, which is generally accompanied by a phase of supernormal heat-production. This phase represents the true "staircase effect" in skeletal muscle. Its duration is much longer than that of the phase of supernormal excitability in nerve, but much shorter than that of the phase of supernormal contractility in cardiac muscle. The "efficiency" of the contraction, as measured by T/H, is noticeably increased as the result of a previous shock at a suitable interval.

7. Curare increases and maintains the contractile power of a muscle placed on a thermopile insulated with shellac.

The expenses of this research have been borne largely by a grant from the Royal Society.

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STUDIES ON THE PHYSIOLOGY OF CAPILLARIES.

II. The reactions to local stimuli of the blood-vessels in the skin and web of the frog.

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The experiments dealt with in the present paper are a continuation of the corresponding studies on the tongue of the frog(1), and the tissue chosen has been selected, partly because some preliminary comparisons showed that the vessels here behaved very differently from those in the tongue, partly also because the anatomy of the nervous system rendered possible a detailed study of the innervation of the vessels of the hind leg. The innervation problems will, however, be reserved for discussion in a separate paper and only incidentally be touched upon in the following pages.

The blood vessels in the skin of the frog, which can be easily observed by reflected light when the skin is strongly illuminated and a part is chosen which is not too strongly pigmented, present a picture very different from that seen in the tongue. The larger vessels, especially the arteries, run on the interior surface of the skin and are only dimly seen. In a large part of the surface the arteries carry venous blood and show up darkly against the white pigment of the ventral surface. These subcutaneous arteries give off numerous branches which pierce the skin, run for a short distance just below the epithelial layer and divide into a very close meshed network of capillaries which are usually open and show a more or less regular current of blood. The veins usually run for a considerable distance at the surface and collect the blood from a large number of capillaries before they pierce the skin. In some places and especially at the ventral surface of the tibia superficial arteries of greater length and diameter can also be observed.

The web consists of two layers of skin which is very thin and, in *R. temp.*, often so slightly pigmented that it can be conveniently observed by transmitted light. Comparatively large arteries and veins run out from the toes between the two layers, but each epithelial surface possesses its own network of capillaries, very similar to that of other cutaneous areas. The capillaries nearest the angle between the middle toes are

usually most suitable for detailed observations on account of their comparatively large distance from those of the opposite surface, and also because the black pigment cells are usually fewer in number than further out.

It has been asserted, especially for the web, that certain capillaries leave the arteries under acute angles and form short connections with diminished resistance between arterioles and venules (W. Jacobj(2), Stromkapillaren), while the larger number, which leave the arteries under more obtuse angles, combine into a network which is only occasionally provided with blood (W. Jacobj, Netzkapillaren, Cohnstein und Zuntz(3), Vasa serosa). Though there are undoubtedly large differences in the resistance of different capillary paths I have been unable to observe any definite distinction between them, and the main point is, as will be shown below, that the resistance and perfusion of any single capillary is extremely variable.

The *methods* employed require very few words since they are essentially the same as those described in the first paper. Medium-sized or small *R. temp.* have been used. They have generally been narcotized with urethane, but most observations have been controlled on curarized or quite normal frogs. Generally the web has been spread by means of small needles on glass plates of suitable size. In some cases I have used glass triangles pushed in between the toes. The two methods have given identical results. The magnifications employed have usually been between 40 and 60 diameters.

1. *The independent reactions of capillaries.* That the capillaries are able to react to local (or general) stimulation independently of the arterial pressure is much less apparent in the case of the web or skin than in the case of the tongue. A local mechanical stimulation at most elicits a response over a very limited area and very often does not elicit any visible response whatever, and the response to most of the chemical substances, which have such a powerful dilator effect on the capillaries of the tongue, is very feeble or altogether absent. Nevertheless, a number of facts can be adduced which show clearly enough that the skin and web capillaries do possess independent tonicity, can contract actively and become dilated by relaxation of their own tonus. A very striking reaction, which I have observed repeatedly on frogs in which the spread web is allowed to dry up somewhat, has been described by Ebbecke(4) (p. 52): "Just after the spreading, the circulation is poor, the arteries are contracted, and many capillaries do not take part at all in the circulation, while others are so far contracted that a corpuscle passes only occasion-

ally. In about half an hour the circulation increases and a number of new, hitherto unobserved, capillaries become visible. The arteries are now very dilated and the flow of blood in the capillaries so rapid that the corpuscles cannot be distinguished. Later on, the arteries contract more and more, while the capillaries dilate and become wider than many of the arteries. The number of visible capillaries is increased three to fourfold and the diameter of the veins is increased in similar proportion. Instead of the rapid continuous flow a distinct rhythm develops, in which the corpuscles are driven forward only during the cardiac systole.... Finally a complete stasis is produced over large areas, and the web appears as a very close network of very dilated capillaries injected with a bright zinnober red dyestuff." Ebbecke's paper contains a large number of valuable observations and is the first in which the significance of independent capillary reactions has been emphasised.

Spontaneous variations in the diameter of single capillaries are of very common occurrence, and it is easy to observe that they are quite independent of the arterial pressure, a dilatation of a capillary often taking place at the same time as a contraction of the artery supplying it. I give as a specially instructive illustration the details of observation made on an arteriole and a small group of capillaries in the web of a frog the sciatic nerve of which had been cut four weeks earlier. Quite similar observations have been made time and again, however, on frogs with intact nerves.

At the beginning of the observation the artery (*A*, Fig. 1) is narrow, the capillary C_1 allows the corpuscles to pass with some change of form, C_2 is dilated, filled with corpuscles, stasis. Somewhat later C_2 is narrow and shows a feeble current, C_1 varies repeatedly from a large diameter allowing the corpuscles to pass freely down almost to obliteration. During these variations C_2 remains for some time closed at x, but lower down a single corpuscle can be seen which is not compressed by the capillary wall. A flow through C_2 sets in suddenly, but the resistance at x remains considerable, allowing the corpuscles to pass in an elongated shape only. A dilatation at x takes place slowly, and after some minutes corpuscles can pass freely along the whole length of C_2. During this process C_1 remains narrow with a feeble current and allows only a small amount of blood to pass through the dilated C_2. A little later C_2 con-

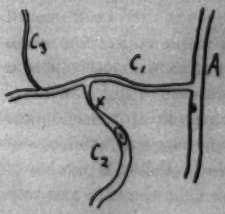

Fig. 1.

tracts and C_1 opens up and shows a rapid current. After weak mechanical stimulation (scratching with a hair) both C_1 and C_2 become somewhat dilated while the artery remains unaffected. After a strong mechanical stimulus applied on the middle of C_2, both C_1 and C_2 contract and become closed and some seconds later the current in A becomes slow and ceases, without the artery being closed within the field of view. After about one minute the current in A is resumed and becomes rapid, and afterwards C_1 and part of C_2 are opened and dilated, while the part directly stimulated, which opens somewhat later, remains so narrow that corpuscles can pass only in a very elongated shape. A little later the peripheral end of C_1 closes up and a capillary C_3, which had hitherto remained closed, opens up. This vessel closes again a minute or two afterwards and the current through C_1 and C_2 becomes rapid.

Weak mechanical stimulation—scratching with a 1–5 mg. hair—has no influence upon wide capillaries, but narrow or closed capillaries are often seen to dilate, though the effect is never considerable. It is only the capillaries directly stimulated or at most at a distance of a few tenths of a mm. which react to this stimulus.

Somewhat stronger stimulation—pressure with a hair of 15–50 mg. or pricking with a fine cactus needle—sometimes causes contraction of the capillary stimulated and one or two neighbouring capillaries. This response can be elicited both from wide and from rather narrow capillaries, but in many cases the effect is local only and in such it may be impossible to decide whether there is active contraction or simple mechanical compression. A very strong stimulus may cause contraction also of the corresponding artery but the time of latency for this reaction is distinctly longer than for the capillary contraction. The response both to weak and to stronger mechanical stimulation takes place after a latent period of 1–5 secs. and lasts usually one to a few minutes.

These observations show very clearly that the cutaneous capillaries can contract and dilate independently of variations in the arterial blood-pressure[1].

An important supplement to this result is furnished by the fact that even considerable variations in pressure have no visible influence upon the diameter of capillaries. Spontaneous variations in the diameter of arteries are of common occurrence in the web, but even when an artery

[1] It is not surprising that the reaction to mechanical stimulation is often difficult to obtain when it is remembered that any stimulus probably affects two distinct sets of receptors, one causing contraction and one dilatation, while the final result must depend both upon the relative sensitivity and upon the local distribution of the receptors.

becomes strongly dilated or contracted the capillaries supplied are not affected except in so far as the velocity of the blood flow through them is altered. When an artery is completely contracted (spontaneously or as a result of stimulation) it can often be observed that the blood flows through a group of its capillaries from a small vein towards another larger vein thus showing that an extremely small pressure is sufficient to keep the relaxed capillaries open. When an artery is very strongly dilated by application of a drop of 0·005 p.c. acetylcholine the dilatation of the corresponding capillaries is scarcely visible though the velocity of the blood flow through them is greatly increased. Not infrequently, however, the application of acetylcholine may *appear* to open up capillaries through which no blood was flowing and which contained only plasma (Zuntz's Vasa serosa). This phenomenon, however, does not necessarily involve capillary dilatation and will find its explanation later in the present paper.

The action of drugs upon the capillaries in the skin and web is much less pronounced than in the tongue. I have never been able to find any distinct effect of 1 p.c. acetic acid which acts so strongly on the vessels of the tongue, but 1 p.c. hydrochloric acid applied in small drops causes dilatation of the capillary or capillaries affected and usually, but somewhat later, of the corresponding artery. 0·5 p.c. nicotine and 1 p.c. iodine produce some dilatation of capillaries. 5 to 25 p.c. urethane produces distinct capillary dilatation and sometimes contraction of the corresponding artery, but the effects obtained are much less striking than in the tongue. When 25 p.c. urethane is applied to the skin the effect can often be observed with the naked eye, the dilated capillaries giving the skin a distinctly red hue showing very nicely against the stratum of white pigment.

In the case of histamine which has been tested in concentrations up to about 5 p.c. no distinctly visible effect upon the capillaries has been found. In a recent paper it has been shown by W. Jacobj(5) that the cuticle of the frog's skin, without being impermeable to such substances as strychnine and adrenaline, offers so much resistance to their passage that their effect is considerably delayed and diminished. W. Jacobj has found further that by treating the skin with alkali or especially with veronal sodium the permeability of the skin is greatly increased. Alkalis and veronal sodium have, themselves, a powerful dilator effect on both capillaries and arteries of the web, but when they are applied in sufficiently weak concentrations the increase in permeability can be demonstrated independently of their dilator effect. I can confirm the observa-

tions of Jacobj which I have utilized to see whether any histamine effect could be found when the permeability of the skin was increased. A drop of 1 p.c. Na_2CO_3, which has no visible dilator effect on the capillaries, was placed on the web and histamine was added until its concentration was at least above 1 p.c. No effect on the underlying capillaries could be detected. Injection of histamine into the web has also been tried with on the whole negative results[1], and although in view of Doi's measurements I do not venture to deny that histamine may show some dilator effect upon the capillaries of the frog's skin I consider this effect to be slight only.

The most powerful dilator stimulus for the capillaries of the web and skin is cutting off the supply of blood. By clamping the femoral artery the current of blood in the web is usually diminished so as to be barely visible in the largest arteries, and when such clamping is maintained for 10–15 min. both arteries and capillaries become slowly dilated and filled with blood. The diameter of capillaries can be observed to increase from about 5 to about 20μ. When the clamp is removed a rapid current of blood will pass through the dilated vessels which after 5 min. are again distinctly contracted and may return to the normal state in 10 min. or less. Other experiments have been made by compressing part of the web in Roy and Brown's apparatus (1) until the circulation was interrupted. On opening after 15 min. the capillaries became strongly dilated while the effect on the arteries was inconsiderable. Cutting off the blood supply in this way for 1½ hours caused the capillaries to relax completely and made their walls permeable so that stasis developed shortly after the opening of the circulation.

As in the case of the tongue it can easily be shown that lack of oxygen has very little to do with this reaction. When the web is exposed to the atmosphere it remains well supplied with oxygen, and the colour of the stagnant blood in the vessels remains arterial throughout. When the web is shut off from the atmosphere by a cover glass the stagnant blood rapidly becomes venous in colour but otherwise the reaction proceeds as in the exposed web[2]. Lack of oxygen does, however, cause some

[1] When a strong solution of histamine is injected a considerable dilatation of arteries may take place and in such cases some dilatation of capillaries is observed also.

[2] It is worthy of note that in these conditions the stagnant corpuscles in the capillaries become practically invisible, because the colour of the reduced hæmoglobin is very faint compared with oxyhæmoglobin. The web therefore appears very pale, and the capillaries may easily be taken to be contracted and empty. When the cover glass is removed the corpuscles become arterialized usually in less than half a minute and the capillaries are seen to be dilated and filled with blood.

dilatation of skin vessels. This can be shown when a cannula is arranged so as to ventilate the lungs artificially. I find that ventilation with hydrogen, which makes the arterial blood in the web turn distinctly blue, produces some dilatation of arteries and also a slight dilatation of capillaries with a distinct increase in flow.

2. *The local reactions of arteries.* While in the tongue of the frog mechanical stimulation of any point will cause dilatation of the artery supplying the point and its surroundings, such a reaction is practically absent in the case of the skin (and web). The arteries themselves are, however, sensitive to mechanical stimulation, and their reactions are on the whole similar to those of the capillaries.

A weak mechanical stimulus such as scratching with a hair of 1–15 mg. or pressing the artery so as to obstruct the passage for a few seconds will generally produce dilatation of the artery at the point stimulated and for some distance both proximally and distally. The reaction usually reaches farther up stream than down stream and may involve a total length of several mm. The time of latency is some 5 to 20 secs., the dilatation begins practically simultaneously over the whole length affected and will last for a very variable period from a few seconds to several minutes. This response is generally easy to obtain when the artery stimulated is in a contracted condition, but very difficult or impossible to elicit when it is dilated. Any part of the superficial arteries in the skin and web can react in this way, but the reaction cannot be obtained from any point along an artery and sometimes a number of trials are required before the vessel reacts.

Contraction of arteries is generally brought about by strong stimuli such as pressing the skin just over an artery with a 50 mg. hair or pricking it with a cactus needle. This latter stimulus will often evoke contraction even when applied at a distance up to 0·2 or sometimes even 0·5 mm. from an artery. The contraction takes place with a latent period of 5–15 secs. and usually almost simultaneously over a more or less considerable distance down stream and especially up stream. Occasionally the portion of the artery to the nearest proximal branch contracts first and a further portion a little later. The contraction often becomes quite complete, and this is the case especially when the artery has been hit and pierced. The contraction usually lasts for a long time up to 10 or even 15 min. When contraction takes place in separate steps relaxation proceeds by the same steps, and the point directly stimulated may often remain contracted for some time after the relaxation of the rest.

As far as my experience goes any part of the superficial arteries can

show the contraction reaction, but it cannot be obtained from all points though the sensitive points are, perhaps, more numerous than those responsible for dilatation. When a highly sensitive point has been found it can be observed that a comparatively weak stimulus may cause contraction over a short distance only while a strong one may act upon the whole visible length of the artery up to 3 or 5 mm. As was to be expected it is generally easier to induce contraction on wide arteries than on narrow, but I have found that it is always possible by powerful stimulation to obtain further contraction so long as an artery is at all open.

The biological significance of this contraction reaction is obvious and it is beautifully illustrated when a cutaneous artery is hit and pierced by a needle. A small drop of blood only is lost before the artery is completely closed, and when it opens again after several minutes this blood has generally formed a clot which prevents further bleeding.

Several drugs, which in the tongue induce a pronounced dilatation of arteries, have a slight and inconstant effect when applied to the skin. Acid (1 p.c. acetic and 1 p.c. HCl) and iodine may sometimes cause dilatation and sometimes contraction, but often these drugs like several others have no effect whatever.

Acetylcholine in ·005 p.c. concentration shows a powerful dilator effect on arteries when applied in drops on the skin, and the effect is equally pronounced on all branches from the largest visible down to the arterioles.

Adrenaline shows a powerful constrictor effect on the larger arteries but does not act upon arterioles and most of the smaller arterial branches. This interesting phenomenon has been studied by placing small drops (·001 c.mm.) of 0·1 p.c. adrenaline on the skin or web just over the superficial branches of arteries. When such a drop is placed over an arteriole it shows no constrictor effect. By following up the artery putting drop after drop over its course a point is finally reached where the action begins. When the drops are placed a short distance apart and half a minute or more allowed to elapse between the single tests it is often observed that the artery begins to contract at a certain distance proximally from the last drop and the contraction spreads slowly in a proximal direction while the artery just below the drop remains open. In the web of medium-sized frogs I have found most of the arteries of less than 0·1 mm. diameter when dilated insensitive to adrenaline, but occasionally even much smaller arteries are distinctly influenced though they will seldom contract completely. In the skin the arterial branches which penetrate from below are usually sensitive to adrenaline, while

the superficial arteries branching from them are not. Exceptions to these rules are not very rare, however.

Jacobj(2) has found that the arteries of the web react to adrenaline only when the substance is applied to the skin in high concentrations (about 0·1 p.c.). After bathing the web with 10 p.c. veronal sodium for some minutes he found the susceptibility of the arteries greatly increased: adrenaline down to ·0002 p.c. being now able to evoke a distinct contraction. I have tested these results and can confirm that the arteries which normally react only to 0·1 p.c. adrenaline will react distinctly to 0·01 p.c. after the web has been bathed for a few minutes with veronal, but all those branches which did not respond to 0·1 p.c. adrenaline before were also completely refractory after the treatment with veronal. It must be concluded therefore that the wall of most smaller arteries and arterioles does not contain the elements acting as "acceptors" to adrenaline.

In connection with the adrenaline experiments a special observation must be mentioned which has been made very often and which is of some importance in judging local capillary reactions both in frogs and higher animals. When part of a small artery branching from a larger vessel is made to contract, without the contraction being complete, as indicated in the diagram Fig. 2, the current of blood through it may seem to cease altogether, and at the same time the corpuscles disappear from the capillaries perfused from the artery. The impression can very easily be produced that the capillaries themselves are obliterated by contraction. A closer inspection shows, however, that the corpuscles are washed out by a current of plasma. The explanation of this phenomenon is to be found in the distribution of the elements in the larger artery, which shows the well-known axial current of corpuscles with a marginal zone of almost clear plasma. When the current in the branch artery is sufficiently reduced by the contraction, the plasma from the marginal zone is simply skimmed off by it. In favourable circumstances it can be observed how at each pulse the column of corpuscles bulges into the mouth of the branch artery and retreats again immediately afterwards leaving only a few corpuscles which become detached and pass swiftly along through the contracted portion of the artery. This plasma skimming is usually very pronounced when adrenaline is applied in small drops to muscle arteries of which all branches, even the smallest, react and show a contraction which in

Fig. 2.

a short time may become complete. The portion of the muscle supplied by the contracting artery becomes blanched and the capillaries often disappear completely from view, while application of adrenaline to capillaries and venules alone shows that these vessels do not react visibly to the substance. Plasma skimming may of course take place to any intermediate extent between giving clear plasma and blood with a normal number of corpuscles, and in the artery skimmed the corpuscle count must rise to a corresponding extent. This phenomenon is probably responsible for many irregularities in the number of red corpuscles observed in counts from capillary blood.

Summary.

The capillaries in the skin and web are contractile and can react independently of the arterial pressure. They show frequent spontaneous, but generally localised, variations in diameter, and they react to various local stimuli. Their reactions, however, differ in several respects from those occurring in the lingual capillaries, and it is desirable to emphasise the quantitative and even qualitative difference in the behaviour of vessels supplying different organs.

The contractile tonus of the cutaneous capillaries is much weaker than that of the lingual capillaries and depends much more upon the blood supply. While the cutaneous capillaries dilate a few minutes after the cessation of the blood flow, the lingual capillaries require a minimum of blood only and remain actively contracted for hours after complete blocking of the circulation.

The cutaneous capillaries may respond to certain stimuli by contraction, to others by dilatation, the lingual capillaries respond solely by dilatation. Localised stimuli have a local effect on the cutaneous capillaries, a more or less widespread effect on lingual capillaries.

The cutaneous *arteries*, when contracted, respond to weak mechanical stimulation by dilatation, sometimes over a considerable length, but a strong stimulus, especially a mechanical insult, evokes a contraction which may also extend over a long distance.

Adrenaline generally affects only the larger arteries, viz. those about ·1 mm. and upwards. Adrenaline causes contraction of all the arteries in frog's muscles, but not of the capillaries.

The blood in a partially contracted arterial branch and the capillaries supplied by it may become anæmic even to the complete disappearance of all corpuscles by skimming off the plasma from the marginal zone of the main artery.

I am indebted to my collaborators, Dr G. A. Harrop and Dr P. v. Liebermann for checking by independent observation most of the results given in the preceding pages.

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ON THE PATH OF CONDUCTION BETWEEN AURICLE AND VENTRICLE IN THE AMPHIBIAN AND REPTILIAN HEART.

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WHEREAS in the mammalian heart the functional connexion between auricles and ventricles is restricted to a single band of modified muscular tissue,—the a.-v. bundle,—in the lower vertebrates, *e.g.* amphibia and reptiles, a condition which is embryonic in mammals persists throughout life, and the connexion is maintained by an annular sheath extending from the auricular canal into the ventricle and lying on the inner surface of the latter. It would naturally be assumed that functional connexion between auricles and ventricles could be maintained by any part of this sheath. On the other hand, it is quite possible that certain portions of the sheath are especially engaged in the conduction of impulses from auricle to ventricle, thus foreshadowing physiologically the condition which finds anatomical expression in the adult mammal.

Various opinions have been expressed on this point by different observers. Gaskell(1) found in the tortoise that the most disturbance was caused when the ventral portion was obstructed. Engelmann(2), investigating the time relations of forward and backward conduction, found a maximum interval of 0·6 sec. and a minimum of 0·25 sec. for the former. For v.-a. conduction he gives as a maximum 0·58 sec. and minimum 0·05 sec. This last figure seems to indicate what Engelmann described as simultaneous rhythm, which in the mammalian heart is spoken of as a.-v. nodal rhythm. Meek and Eyster(3) investigating the question by the galvanometer, confirmed Gaskell's view that the ventral portion of the ring is the most important channel. On the other hand, Külbs(4) maintained that the lateral portions of the ring were the most important channels, largely on the grounds that it is only in these parts that the fibres pass down for any distance into the ventricle, being interrupted in front by the vessels and behind by the valves. Laurens(5) confirmed Külbs. He considered that the left side was the chief path of conduction. A small portion of the left part of the ventral segment may also be brought into play. Injury of these parts leads, according

to him, progressively to slowing of a.-v. conduction, dropping of ventricular beats, incomplete block, and v.-a. rhythm. Judging from Laurens' tracings, it would seem that he was dealing rather with a complete dissociation than a v.-a. sequence. T. Lewis(6) showed that the first part of the ventricle to receive the excitation wave was the inner surface. This would correspond to the anatomical relationship of the conducting sheath. According to Skramlik(7) a.-v. conduction is effected chiefly by the posterior dorsal part of the ring, while v.-a. conduction is carried out by the septum.

I have re-investigated the question by a different method.

Method. The a.-v. ring was tied in segments with strong silk and the immediate and later effects noted. The method seems to me to possess certain advantages which others lack. For instance, perfusion can be kept up without any leakage of consequence,—one can adjust the pressure to the leak—and perfusion is of importance for the observation of rhythm changes some time after operation. Scissors cuts, which have been favoured by some, do not admit of this, while local heating and cooling, which can only be of use in observing results for quite limited periods without perfusion, would be rendered practically useless if this were kept up, for without wide destruction the inner layers would be very difficult to cool or heat sufficiently for blockage of conduction. If a suitable clamp could be devised for pinching limited parts of the ring, it should prove of use, and this as yet I have not succeeded in doing, as even with pointed ends for fixing the limits it is difficult to get just that portion of the ring one wants. Transfixion with a needle, even in the small heart of the frog, is, with care in keeping to the a.-v. groove, reliable for picking out a particular portion.

The a.-v. ring is considered in four chief segments or quarters, a ventral, a dorsal, and two lateral. But some portions were ligatured which included parts of two segments. For instance, a combination ligature including portions of the left lateral and ventral segments is that which has given most interesting results, results which do not accrue from annulling either separately. The ventral segment, limited by the bulbus on the right and by the intermediate part on the left, is very small. The bulbus was generally included in the right quarter by tying off the thread underneath it so as to leave an exit for the fluid, but it was soon found that the portion corresponding to the bulbus had no influence on conduction, and in the later experiments it has been left aside without touching.

Suppressing function in certain parts of the ring by the method

described enables one to study the properties of other parts several hours later, without the fallacy of having to attribute failure to assume new functions or to arouse a dormant one to adverse conditions and defective metabolism. A record from a heart some hours after injury generally shows that whatever ventricular activity exists in it is due to auricle or sinus influence, automaticity in the ventricle as a result of the mechanical stimulus of the ligature usually subsiding after a time or appearing periodically. This is important, for otherwise it would be difficult to determine what might be idioventricular beats and what conducted stimulus beats. Sometimes a ligature produces no ventricular disturbance which could mask a block, and theoretically one should avoid it by keeping rigidly to the groove or even above this, but it has been found that the high ligature has to include a wider area of tissue than a low one to give a similar result in a certain region.

I have grouped the experiments in two tables, the first table dealing with conduction from auricle to ventricle, the second table with reversed conduction from ventricle to auricle. Each experiment numbered in the table is a sample of a number of experiments of the same type, the actual number performed being given in the last column.

It is evident from these results that damage to a left ventral segment causes more disturbance of the conduction from auricles to ventricle than injury to all the other parts taken together. The recovery of function after the former injury is usually slow and incomplete, whilst there may be complete and fairly rapid recovery from damage to other segments, and these are quickly corrected and rarely permanent. It is possible that in the latter case there may have been indirect mechanical injury to the left ventral path. We cannot exclude the possibility of some reflex influence of the operative procedure. Thus, the mere insertion of a needle into the left ventral region may cause the ventricle to stop beating for a minute or more. In this case we cannot conceive that a thrust of a fine needle could destroy anatomically the whole conducting path. The results must be either due to the transmitted effects of the injury, or to the prick of the needle evoking an axon reflex from the heart ganglia which inhibits the conducting powers of the whole ring of conductive tissue. But it must be admitted that the most facile conducting part of the funnel and the one ordinarily occupied in the transmission of the excitatory wave is a limited one, located in the left ventral region.

The microscope reveals that the ventral wall of the atrio-ventricular funnel is much thicker than the rest and penetrates further into the

TABLE I.

No.	Animal	Segment tied	Early effects	Later effects	Duration of observation	Level of ligature	Number of experiments
1	Frog	Left	Extra beats ventricle	None	½ hour	Below	3
		Deeper, same side	Block 1 min.	(1) V. 1–A. 4 (2) V. 1–A. 2 permanent	3 hours	Groove	
2	Frog	Dorsal	Very irregular	None	—	—	6
		Dorsal, deep	Reversed rhythm 6 beats	None	½ hour	Below	
		Right	Paraasystole	V. 1–A. 2 two mins. then 1–1	10 mins.	Below	
		Ventral	Complete block	V. 1–A. 6 three mins. later 1–4	1 hour	Groove	
		Left	A few excited ventricular beats	Complete block	2 hours	Groove	
3	Frog	Right	Reversed beat?	None	½ hour	Groove	6
		Dorsal	Irregularity	V. 1–A. 2 then slow 1–1	10 mins.	Groove	
		Ventral	None	None	10 mins.	Above	
		Left	Dissociation	V. 2–A. 1 irregular, then block for 10 mins. with idiopathic ventricular beats, then permanent stop	2 hours	Below	
4	Frog	Left and dorsal	Very irregular	V. 1–A. 3 later 1–1	½ hour	Groove	6
		Rest of left and ventral	Complete block	V. 1–A. 3 permanent	2 hours	Groove	
5	Tortoise	Right	None	None	—	Groove	3
		Left	V. 1–A. 2	V. 1–A. 2	10 mins.	Groove	
		Ventral	Irregularity	V. 1–A. 7 permanent	20 hours	Below	
6	Tortoise	Left	V. 1–A. 2	V. 1–A. 2	½ hour	Groove	3
		Ventral	V. 1–A. 3	V. 1–A. 4	1 hour	Groove	
		Right	Very irregular	Long stoppage followed by occasional beats	1 hour	Groove	
7	Snake	Right	None	None	½ hour	Groove	3
		Left	Irregularity	V. 1–A. 3 then V. 1–A. 2	½ hour	Below	
		Ventral	Complete block 5 mins.	Occasional grouped ventricular beats	1 hour	Groove	

ventricular muscle. It is almost circumscribed as a separate bundle, but has remains of the septum, some threads of the other portions, and the Bidder ganglion cells in close relation to it. It is pushed slightly to the left by the arteries. The culmination of the auricular contraction wave in the lower and anterior portion of the septum and funnel, and the transmission to the ventricle by a restricted bridge of tissue, fit in with our ideas of the spread of the contraction wave from the sinus forward and downward.

TABLE II. *Reversed transmission.*

No.	Animal	Segment tied	Stimulation	State of heart	Reverse conduction	Forward conduction
1	Snake	None	Apex (3)	Stannius-quiescent	No	—
		"	Apex after interrupted current	" "	Yes	—
		Left	Apex	" "	"	—
		"	Auricle	" "	—	No
2	Frog	None	Apex after vagus stimulation	Ordinary rhythm	"	Yes
		Left	Apex	Stannius-quiescent	No (occasional?)	—
3	Snake	Ventral and left	Apex	Not stannius. Block	Yes	No
4	Tortoise	None	Apex	Ordinary rhythm	No	Yes
		Right and dorsal	Apex freely	" "	Yes	"
5	Frog	None	Apex	Ordinary	No	"
		Dorsal	Apex after vagus stimulation*	Stannius-quiescent	Yes	"
		Left	Apex	Stannius *not* quiescent	"	"
6	Frog	Ventral	Apex	Stannius-quiescent	"	No
7	Frog	Right, left and ventral	Apex freely	" "	No	"

* Vagus stimulated before application of ligature.

It is interesting to note in these experiments that reversed transmission can occur in a portion of the ring which fails to transmit from above. It can be induced by stimulation of the ventricular apex, after blocking the a.-v. impulses by ligature of the left ventral segment. In this case, stimulation of the ventricle gives v.-a. whereas stimulating the auricle is followed only by an auricular beat.

Reversed conduction is not a normal function of any part of the ring, though it may be induced in different ways. It may occasionally be evoked by the mechanical injury caused by placing the ligatures. I have noticed it frequently when the ligature was placed dorsally, especially when fairly low down in the ventricular tissue.

I am inclined to conclude from these experiments that the atrio-ventricular ring is equally facile or equally difficult in all parts for the reversed v.-a. transmission, and that any segment may by suitable

means be open to upward transmission, even when the ordinary a.-v. impulses are blocked. Among these means may be mentioned electric stimulation of the vagus as well as of the heart itself.

Conclusions.

In the amphibian and reptilian heart the atrio-ventricular funnel does not conduct as a whole. Every segment of it, with the possible exception of the dorsal, is capable of conducting. There is least resistance to auricle impulses in a segment which is placed ventrally and to the left, and which is comparatively small. Corresponding to this site there is a thick mass or accumulation of muscle on the ventral wall of the funnel, which pushed to the left penetrates more deeply into the ventricle than the remaining portions of it. This, with the experimental results, is suggestive of a restricted bridge which ordinarily conveys auricular impulses; these, however, may be conveyed along other channels when this bridge is occluded, the impulses having to overcome a varying resistance in these channels.

Reverse conduction has no smooth path open for it; a certain degree of resistance has to be overcome in each instance in which it occurs. This resistance seems to be more or less uniform in all those segments which are capable of downward conduction, and therefore the upward and downward paths are not necessarily one and the same when the two rhythms alternate.

Resistance to upward conduction may be reduced by electrical stimulation of the vagus or of the heart directly.

Automatic properties in the ventricle have been in these experiments exhibited more markedly by the dorsal portion and sides of the ventricle base than elsewhere, which indicates that these are not correlated with the chief conducting function.

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Spontaneous movements of blood-vessels. By R. J. S. McDowall. (*Preliminary Communication.*)

The existence of rhythmical variations in the circulation has been known for many years, but up to the present it has been generally assumed that these changes are of central origin. Such rhythmical movements have been observed by many early investigators. More recently it has been noted (Kravkov) that similar changes occur in the arteries of mammals and they occur quite independently of heart or central nervous system, while graphical records of similar movement in excised rings or spirals of arteries, especially after the action of adrenalin, have been obtained by other observers.

During perfusion of the pulmonary circulation with Ringer-Locke fluid (without bicarbonate) waves of contraction and relaxation have been found to occur frequently in young and robust rabbits and cats, and by connecting the inlet tube to a water manometer and piston recorder graphic records of such waves are obtained if the pressure be suitably adjusted. The waves are not always regular; and in different animals they may vary from one or two to ten per minute, continuing it may be for several hours. Pituitary extract, adrenalin and temporary distension of the lung alveoli have a stimulating effect.

A tracing showed that each contraction caused complete occlusion of the pulmonary circulation, as evidenced by cessation of flow from the exit tube. The waves are regular, at the rate of one and a half per minute and continued for three and a half hours.

The experiments are being extended to the systemic circulation, in which the author has already found similar waves. It seems certain that the existence of such spontaneous contractions and relaxations will have to be considered in questions regarding variations of pressure hitherto attributed to the vasomotor centre.

Electrical variation of the pancreas. By G. V. Anrep and I. de Burgh Daly.

Experiments were performed upon cats under chloroform and ether or urethane anæsthesia. The intestines were removed from as high as possible without injuring the pancreas and secretin prepared in the usual manner; in some experiments the secretin was prepared from another animal.

In the earlier investigations two non-polarisable electrodes were placed upon the pancreas, one as near as possible to the duct, the other on the splenic end. The electrodes were connected to a Broca or Einthoven galvanometer, the string tension of the latter being greatly reduced in order to increase the sensitivity. The galvanometer showed rhythmic deflections which were found to be due to respiratory movements and movements of the remaining portion of the intestine. The respiratory deflections were abolished by opening the chest, cutting the phrenic nerves and reducing artificial respiration to a minimum compatible with sufficient lung ventilation. The intestinal deflections could not be completely abolished but were greatly reduced by surrounding the pancreas with sheets of rubber.

Injection of secretin into the jugular vein, when the electrodes were placed in the position described above, with the exception of a few cases did not give any electrical variation although pancreatic secretion was produced. When deflections did occur the duct became positive to the splenic end.

In later experiments, a cannula in the pancreatic duct was connected to a T-piece non-polarisable electrode and the pancreatic juice allowed to flow over the electrode. Injection of secretin into the jugular vein then caused a well-marked variation in which the duct was positive to the surface of the pancreas.

The rate of secretion of the pancreas was measured by observing the flow along a capillary tube and signalling the amount on the photographic plate which registered the electrical deflection. The curves denoting the rate of secretion were calculated and superimposed on the plate with their correct time relations. The electrical variation always preceded the secretion, and was always observed when secretion was present.

It has been shown by Hill and Brown(1) that the movement of salt solution past silver chloride electrodes produces an E.M.F. The non-polarisable electrodes used throughout these experiments were made of kaolin and ·9 p.c. NaCl and did not show the phenomenon described by

Hill and Brown, therefore the pancreatic secretion flowing past the electrodes would not be the cause of the electrical variations obtained. To test this point further we occluded the pancreatic cannula distal to the electrode; on injecting secretin there was an electrical variation although there was no movement of juice past the electrode.

Three successive injections of secretin 2, 4 and 8 c.c. at signals. Broken curve = electrical variations; plain curve = rate of pancreatic secretion. Each interruption on electrical variations represents 25 secs. The smaller electrical deflections are due to intestinal movements. Broca galvanometer of 968 ohms. 1 millivolt = 600 mm.

Injection of secretin produces a fall in blood pressure, therefore the electrical deflections might be due to a vascular change. We obtained no electrical changes by independently altering the blood pressure from 40 to 200 mm. Hg.

We conclude that the electrical variations of the pancreas observed after the injection of secretin are due to the increased activity of the gland.

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Some effects of hot baths on man. By H. C. BAZETT and J. B. S. HALDANE.

Ten experiments were made on the effects upon four men of immersion up to the neck in a bath in which the water was kept circulating at a constant temperature. By this means the body temperature can be kept steady or made to rise at any desired rate. A bath between 35° C. and 36·5° C. feels cool, and the subject's temperature may vary slightly or remain steady. Between 36·5° and 37° it felt most comfortable, but the body temperature might rise to about 37·5°. In a bath above 38° C. the body temperature rose to a level 0·2° or less above that of the bath. Urine and rectal temperatures rarely exceeded mouth temperatures by 0·1°, so the latter were mainly recorded.

In baths below 37° respiration was unaffected, pulse rate rose slightly, systolic and diastolic arterial pressures fell 10 to 20 mm. of Hg, and venous pressure seemed to be increased. There was a striking diuresis reaching a maximum rate (in one case of 750 c.c. per hour) during the second half hour of immersion. The normal rate was re-established by H. C. B. after three to four hours, and excretion of about 1200 c.c. The urine was pale and neutral to litmus. The excretion of acid (titrated to phenol-phthalein) and NH_3 per hour remained fairly steady though their concentrations fell. Chloride concentrations varied between ·033*N* and ·080*N*. Though these values are lower than those of plasma it seems likely that the extra urine represents tissue fluid squeezed into the veins by the pressure of the water. It was not due to absorption of water, as no weight was gained.

In hotter baths sweating began at a mouth temperature of 37·2°. During rapid rise of temperature hyperpnœa occurred. Its intensity varied with the rate of rise rather than the temperature. Thus J. B. S. H. with a temperature of 37·2° rising at ·13° per minute breathed 27·3 litres per minute against a normal 6. The respiratory quotient was 1·3, and the alveolar CO_2 had fallen from 38·7 to 25·6 mm. The hyperpnœa was accompanied by faintness, mental confusion, and tingling; which were relieved by breathing mixtures containing either 8·5 p.c. CO_2 with 14·2 p.c. O_2, or equal volumes of air and O_2. They were therefore probably due to oxygen want in the tissues caused by over-stability of oxyhæmoglobin resulting from acapnia, as in voluntary forced breathing. When the temperature became steady all these symptoms abated. Thus with a steady body temperature of 38·6° J. B. S. H. breathed 12·7 litres per minute, with R.Q. 0·81 and alveolar CO_2 30·0 mm.

During severe sweating and hyperpnœa the diuresis was less intense and prolonged, but the urine was very alkaline, that of H. C. B. reaching the "record" C_H of $10^{-6.0}$. It contained no NH_3, much bicarbonate, and some acetone bodies. With less hyperpnœa the hourly output of acid and NH_3 fell, but did not vanish. Sweat collected from H. C. B. had a P_H of 7·9, so no acid was lost through the skin. It is clear that heat makes the respiratory centre more sensitive to CO_2, and the resulting acapnia causes changes in the blood, nervous system, and urine precisely similar to those found in voluntary forced breathing.

Hæmoglobin estimations on H. C. B. showed a rise of 1 to 1·5 p.c. in neutral baths, and with rapid sweating at a rate of 0·7 to 1 kilo per hour a rise of 10 to 16 p.c. at a time of day (12—4 p.m.) when his concentration is normally fairly steady. There was thus presumably a fall in his blood-volume. If there was much loss of fluid (in H. C. B. after the loss of 2 kilos in weight) restlessness and a rapid small pulse might supervene. Otherwise the circulatory changes resembled those in neutral baths, except that the pulse rate and pulse pressure rose with the body temperature. Symptoms of circulatory failure seemed to occur more readily in an older subject (B. M., aged 54).

Our thanks are due to Prof. B. Moore, Dr J. S. Haldane, and Dr E. F. Adolph for assistance, and to the Medical Research Council for a grant to meet expenses.

The effect of thyroxin on growth in white rats. By A. T. Cameron and J. Carmichael.

We have recently suggested that decrease of growth-rate in growing rats, hypertrophy of heart, liver, kidneys, and adrenals, and decrease of growth-rate of thyroid can be used as tests for preparations alleged to be the essential chemical compound secreted by the thyroid gland (*Physiol. Proc.*, Nov. 20, 1920; *Journ. Biol. Chem.*, 45, 69. 1920).

Thyroxin produces these effects when fed by mouth. Quantitatively, compared on a basis of iodine content, the effects of thyroxin are distinctly less than those of desiccated thyroid. This is probably due to bacterial decomposition; thyroid acts as a shield.

The hypertrophy of heart and lymphatic tissue resembles that observed in cases of hyperthyroidism.

The details of our experiments will be published shortly.

Observations on "second wind." By N. W. MacKeith, M. S. Pembrey, W. R. Spurrell, E. C. Warner, and H. J. W. J. Westlake.

Former experiments(1) upon "second wind" showed that the condition is accompanied by a fall in the percentage of carbon dioxide in the alveolar air as compared with the samples taken during dyspnœa. The present experiments have been carried out in an attempt to determine the factors concerned in the compensation.

Alveolar Air. The following are samples for the four subjects:

	Alveolar air in vols. per cent.			
Subject	Rest	Dyspnœa	"Second wind"	"Breaking point" at rest
W.R.S.	4·15	5·43	4·91	5·8
E.C.W.	5·97	6·38	5·71	6·2
N.W.M.	5·61	6·45	6·24	8·8
H.J.W.J.W.	5·71	6·13	6·04	—

Urine. The changes in the volume, acidity and ammonia were studied; tea (500 c.c.) was taken as a diuretic in order to ensure an adequate sample and controls at rest were made.

Volume. Very little urine could be obtained during the latter part of dyspnœa and this condition of apparent anuria persisted during "second wind." On the other hand if the exercise was slight, slow running at the rate of four miles per hour, the normal diuretic volume curve was obtained, the alveolar carbon dioxide was steady, there was no dyspnœa, and no "second wind." The influence of the cutaneous circulation, blood-pressure and adrenalin upon the secretion of urine during exercise is under investigation.

Acidity. The urine was titrated against $N/100$ NaOH to a P_H 7·4, with phenol red as an indicator; all the samples of urine were collected and analysed under liquid paraffin.

The bladder was emptied and then running started until dyspnœa was definite at about a quarter of a mile, the urine was now collected, running restarted and continued until the onset of "second wind," when the bladder was again emptied. The acidity per 10 c.c. of the sample during dyspnœa fell in a manner similar to the normal diuretic action, whereas during "second wind" it rose rapidly.

Ammonia. The outputs of ammonia per 10 c.c. and per minute respectively closely correspond with those of acid; during dyspnœa it falls and during "second wind" rises rapidly per c.c. of urine.

Sweat. The onset of sweating closely corresponded with the establishment of "second wind." Sweat collected in vests free from chloride gave definite amounts of lactic acid, as estimated by Ryffel's method. The content of chloride was taken as an indication of the amount of sweat present and the lactic acid/chloride ratio was determined for comparison with the results obtained during sweating at rest in an electric bath.

		Chloride	Lactic acid	Lactic acid / Chloride
E.C.W.	21. ii.	183·18 mg.	56·23 mg.	·306
N.W.M.	22. ii.	251·34	65·3	·26

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The experimental production of thyroid hyperplasia in dogs. By EDWARD MELLANBY and MAY MELLANBY. (*Preliminary Communication.*)

In making an examination of the thyroids of dogs used by us in connection with work on rickets and the production of defective teeth respectively, several facts came to light which promised to be of interest. As the investigation has been up to the present almost entirely statistical, only those points which stand out prominently can be mentioned with any certainty.

1. When puppies received cod liver oil as the only fat in the diet, the thyroids were small and normal in appearance. With other fats the appearance and size of the glands varied, but there was always some hyperplasia as compared with those of the cod liver oil puppies. When butter was the fat eaten, the hyperplasia of the thyroid glands was especially large. The average weight of the thyroids of 12 puppies receiving cod liver oil was 0·586 gram, while that of 8 butter-fed puppies was 2·77 grams, *i.e.* a fivefold increase. The increase in size was accompanied by equally striking changes in histological appearance. These latter were not constant, the hyperplastic changes in some cases affecting to a greater extent the secreting cells of the alveoli and in other cases the intervesicular cells.

2. Increasing the fat of the diet from 10 to 20 grams per diem intensified, often to a remarkable degree, the hyperplasia of the thyroids. Five animals receiving 20 to 25 grams of fat per diem developed thyroids whose weights were 4·181, 7·043, 6·929, 13·388 and 10·538 grams respectively, *i.e.* on the average over 14 times the weight of those of the

cod liver oil puppies. It may be added that the size and weights of the puppies with these large thyroids were not above the normal, nor were they older than the other animals.

3. The size and appearance of the thyroid gland varied with the energy of the food eaten and the opportunity the animal had of getting rid of this energy by mechanical effort or by living out of doors. Thus confinement and living indoors, together with a large diet, especially one containing abundant fat (other than cod liver oil), produced great hyperplasia of the thyroids. Reducing the diet and especially the fat quota, affording the opportunity for running about in the open air and so increasing energy expenditure relatively to the intake, produced smaller and more normal glands.

Cholin as a precursor of creatin. By W. F. SHANKS. (*Preliminary Communication.*)

In considering the possible sources of guanidin and methyl-guanidin in the body, Noël Paton referred (Presidential Address to the Physiology Section of the British Association at Bournemouth, 1919) to the work of Burns which had demonstrated an increase in the guanidin content of the hen's egg during the first twelve days of incubation, at which date creatin first appears. A probable source of the guanidin was pointed out in the cholin of the lecithin which is decomposed to yield phosphoric acid for the formation of nucleins and of bone.

The investigations of Riesser(1) had supported this view by showing an increase of the methyl-guanidin-acetic acid (creatin) in the muscle of the rabbit after the administration of cholin. This worker injected cholin subcutaneously over a short period and found that the creatin content of the muscle was raised above the normal previously determined by him. Baumann, Hines and Marker(2) state that by perfusing cholin and urea in the dog they got an increase of the creatin in muscle.

Guggenheim and Löffler expressed the opinion(3) that cholin undergoes some change in the body since only minimal quantities are excreted in the urine.

The following experiments are an extension of Riesser's work by a different method.

A rabbit was anæsthetised with ether and one gastrocnemius removed. A weighed quantity of cholin hydrochloride in normal saline was then slowly run into the jugular vein. Light anæsthesia was maintained for three hours after completion of the injection and then the second gastrocnemius was removed and the animal killed.

The creatin in the two muscles was estimated by the method described by Baumann(4), the analysis being carried out side by side. The water content and total nitrogen of each was also determined.

Results are appended:

No.	Weight of rabbit	Substance administered	% creatin in dry muscle		Remarks
			Before	After	
2	2600 gms.	Cholin ·262 gm.	1·88	2·01	—
3	2100	„ ·550	1·94	1·89	—
4	2500	„ ·637	1·88	1·94	—
5	1600	„ ·800	1·93	2·12	—
6	2100	„ 1·103 gms.	1·70	1·73	Death about one hour after completion of injection
11	3500	„ 1·150	1·87	1·98	—
7	1750	Normal saline 30 c.c.	1·81	1·75	—
8	1800	Normal saline 30 c.c.	1·86	1·80	—
9	1850	$BaCl_2$ ·0035 gm.	1·85	1·93	—
10	2050	$BaCl_2$ ·0030 gm.	1·90	1·91	—

Controls with normal saline only and with $BaCl_2$ were done, the latter being used on account of its somewhat similar action on visceral muscle.

The average of the experiments in which cholin was administered (excluding No. 6) gives:

	Before	After
Creatin	1·90 %	1·99 %

The average of the non-cholin experiments gives:

	Before	After
Creatin	1·85 %	1·85 %

Conclusions.

(i) Intravenous administration of cholin tends to raise the creatin content of muscle.

(ii) This is independent of any change in the water content.

(iii) The average dose of cholin given was about 0·3 gm. per kilo of body weight, the average gain of creatin in three hours was probably roughly about 0·08 gm. per kilo, so that these results are not incompatible with Riesser's theory of the conversion of cholin into creatin by linking with urea (*loc. cit.*).

I wish to thank Professor Noël Paton for his assistance and also Dr Burns for the performance of some of the experiments.

The expenses have been partly defrayed by the Carnegie Trust.

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The application of the results obtained in experiments on the hyperplasia of dogs' thyroids to the treatment of exophthalmic goitre (Graves' disease). By EDWARD MELLANBY and MAY MELLANBY. (*Preliminary Communication.*)

We were impressed by the similarity in histological appearance between some of the hyperplastic thyroids of the dogs, whose production is described in another communication, and those of patients suffering from exophthalmic goitre. We decided, therefore, to try the effect of dieting patients suffering from this disease along the suggested lines.

It was first observed that, when butter was added to a good normal diet, all the symptoms were intensified. The metabolism was increased to such an extent that the patient lost weight, and the tachycardia and nervous condition were more pronounced. The diet was then arranged so that it contained only a small amount of fat and to this was added 15 c.c. of cod liver oil per diem. The foodstuffs eaten were separated milk powder, porridge, bread, green vegetables, eggs and lean meat. Although this diet on combustion in the body supplied much less energy, the metabolism of the patients was reduced out of all proportion and they now gained in weight. In some cases the gain in weight was astonishing, as for instance:

G.	gained	13	lbs in	15 days.
T.	„	9	„	14 „
M.	„	6½	„	14 „
B.	„	4½	„	20 „

During this period of reduced metabolism, the other symptoms of Graves' disease were greatly mitigated, the tachycordia and restlessness being especially modified in their intensity. Working on the assumption that the action of cod liver oil in the case of the puppies' thyroids was wholly or in part due to its iodine content, 6 to 9 grains of potassium iodine were in some cases given to the patients.

After a period of this treatment resulting in reduced metabolism, the weight of the patient became stationary or fell slightly.

The results so far obtained suggest that the same physiological laws apply in the production of certain thyroid abnormalities alike in dogs and human beings and that there is good hope of a curative treatment of exophthalmic goitre being developed by the direct application of results obtained in experimental animals along these lines.

Some observations on the pulmonary circulation. By F. A. Bainbridge and S. W. F. Underhill.

This series of experiments deals with the effects produced on the circulation by ligature of one of the main divisions of the pulmonary artery. The experiments were carried out on cats anæsthetised with ether; and the branch of the pulmonary artery chosen was invariably the left, as it is the more accessible. The pressure at the root of the pulmonary artery was determined by the method recently introduced by Sir Sharpey Schafer. Two sets of experiments were performed.

(*a*) *Chest open.* Ligature of the left pulmonary artery causes an immediate rise of pressure in the main pulmonary artery: the rise as a rule is from 30–50 p.c. of the original pressure. It is usually maintained for 10–20 mins.; after this time the pressure gradually falls and may almost reach the level existing before the application of the ligature. The gradual fall of pulmonary pressure is sometimes associated with a corresponding fall of aortic pressure, but it may occur without any change in the latter.

Protocol of a typical experiment:

Time p.m.	Remarks	Pulmonary pressure mm. citrate solution Sp. gr. 1024	Aortic pressure mm. Hg
4·4	Chest open. Artificial respiration	325	75
	Left pulmonary artery tied	440	—
		460	72
4.12	—	420	73
4.19	—	425	70
4.25	—	395	70
4.33	—	350	70
4.38	Blood sample (1 c.c.) removed	345	60
4.42	Ligature released	275	55
4.56	—	305	65

Ligature of the left pulmonary artery causes almost no change in the aortic pressure, the pulse rate or the output of the heart. Further, if care is taken to maintain fairly vigorous artificial respiration, the arterial blood may be as fully saturated as in the normal animal.

If the ligature is released, the pulmonary pressure falls, either to its original level, if the rise has been maintained, as occurs in a short experiment, or to a level which is lower than the original, as in a prolonged experiment, where a gradual fall has occurred after the rise. The aortic pressure remains steady, or may rise.

(*b*) *Chest closed.* In some experiments, after ligature of the left pulmonary artery, the chest was closed, the air was sucked out of the pleural cavities and the animal was allowed to breathe naturally. As

a control, a cannula connected to a water manometer was passed through the chest wall: and, before allowing natural respiration, it was ascertained that the normal negative intrathoracic pressure had been re-established. In these circumstances the animals showed considerable dyspnœa and, in three out of four experiments, death occurred from respiratory failure within half an hour. Post mortem, the appearance of the left lung showed a striking contrast to that observed when the chest was open. In the latter case with the lungs artificially ventilated, the lung, after ligature of the pulmonary artery, is obviously blanched as compared with the normal lung. Where, however, death occurs with the chest closed, the (ligated) lung appears as full of blood as the sound lung. On section blood oozes from it, and microscopic sections show that its capillaries may contain as much blood as those of the sound lung.

During natural respiration the pressure on the outer walls of the capillaries must be negative: and it is suggested that, as a result of the respiratory movements, blood is sucked from the left auricle into the pulmonary capillaries, where it stagnates, thereby lessening the volume of the circulating blood.

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(1) Sharpey Schafer. Quart. Journ. Exp. Physiol. 12, 133.

Engelmann's evidence for the independence of excitability and contractility in heart muscle. By T. Lewis and A. N. Drury[1].

In his paper "Ueber die bathmotropen Wirkungen der Herznerven," *Archiv f. Anat. u. Physiol.*, 1902, Physiol. Abth., Suppl. Bd. I.), Engelmann brings forward evidence to show that the two properties known as excitability and contractility are wholly independent functions of heart muscle. This, the main conclusion of his paper, has received a wide measure of acceptance; his most striking evidence consists of a series of four myograms (Figs. 9–12) from the frog's auricle. The auricle was stimulated rhythmically by shocks reaching it at a rate of one per second; the auricle responded to alternate shocks only. The vagus was now stimulated reflexly, whereupon the strength of the auricular beats declined while the auricle responded to each stimulus reaching it. Engelmann interprets this change as due to a decline of contractility (negative-inotropic nerve-influence) with a rise of excitability (positive-bathmotropic nerve-influence).

The phenomenon witnessed by Engelmann has been recently investigated and its true explanation proves to be much more simple. When

[1] Working on behalf of the Medical Research Council.

the auricle (in the case of our experiments, the dog's auricle) is driven at a sufficient speed, it fails to respond to alternate shocks; these shocks fall, as we are able to show, a little before the ending of the absolute refractory periods of the preceding systoles. If now the vagus is stimulated, the systoles of the auricle are not only reduced in amplitude but they are also shortened, the corresponding refractory periods are reduced (see *Proc. Physiol. Soc.*, Dec. 18, 1920) and the auricle at once responds to each stimulus (Fig. 1). It is not, as Engelmann would have had us believe, a rise of excitability which is responsible for this reaction, but an earlier development of the responsive stage, consequent upon the shortening of systole. The vagus depresses all the properties of the muscle which it influences, and apparently simultaneously, but

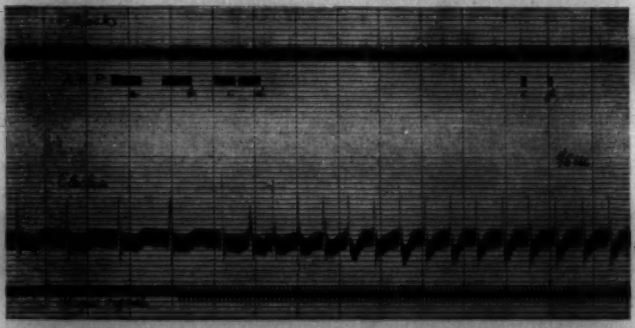

Fig. 1. The top record is of the shocks entering the auricle (rate 500 per minute), the middle record is an electrogram taken from the auricular muscle some distance from the point stimulated. A.R.P. = absolute refractory period represented diagrammatically. Before vagal stimulation the auricle is in a stable condition of half-response; shortly after and as a result of vagal stimulation it breaks into complete response. The shocks were well above threshold value throughout. The A.R.P., during the period of half response, exceeds slightly the intershock interval (as represented at *a* and *b*). At a late stage of vagal stimulation the A.R.P. is measured at about ·02 of a second (as represented at *e* and *f*). The appearance of complete response evidently results from shortening of the A.R.P. (as represented at *c* and *d*).

by depressing them it renders the muscle responsive to shocks at an earlier phase of the cycle.

Engelmann used threshold shocks; the phenomenon is equally well displayed when much stronger shocks are used.

Thus Engelmann's most striking evidence for the independence of the properties excitability and contractility in heart muscle must be disallowed; being disallowed, the remaining evidence appears to us insufficient to establish his main contention.

The tetanic nature of the voluntary contraction in man. By A. V. Hill.

By the aid of the hot wire sphygmograph it is possible to record in the string galvanometer the vibrations of the muscles of the human body. Piper(1) showed that a human muscle provides electric changes at the rate of about 50 per second when thrown into voluntary contraction. The rapidity of contraction and relaxation in a muscle twitch at 37° C. is so great that such a rate of stimulation might be expected to yield only an incomplete tetanus, *i.e.* a large movement with small superimposed oscillations; experiment shows this to be the case.

If a "glycerine tambour," or a large plain tambour with a tight membrane, be pressed upon any muscle in the body and joined to a pipe connected to the hot-wire sphygmograph, the oscillations of the muscle cause vibrations of the air inside the tambour and pipe, which cool the hot-wire and can be recorded on a string galvanometer. The simplest method of showing the effect is to take the tambour between the first and second fingers, and to press gently upon the surface with the thumb. However gently one may press, the oscillations of the muscles are communicated to the galvanometer and are shown on the photographic record, at the rate of 40 to 50 per second. The best results are obtained by slowly pushing in with the thumb, as in *A*, *B* and *D* in the figure, but similar records can be made by maintaining a steady pressure on the tambour. The vibrations are not instrumental in origin, as is shown by the facts (i) that different subjects give different rates of vibration, see *e.g.* (*A*) and (*B*) in the figure; (ii) that the same subject gives the same rate with different tambours, see *e.g.* (*A*) and (*D*); and (iii) that the vibrations of the tambour itself are at quite a different speed, see *e.g.* (*C*) where the tambour was struck and its vibrations recorded. The oscillations are not due to "creaking" of joints, as is shown by the facts (i) that the tongue pressed against the tambour sets up vibrations at the same rate, and (ii) that when the tambour is tied directly on to such muscles as the biceps or the calf, the same effect is shown. It would appear that the oscillations observed represent a true vibration of the muscle fibres themselves. The records are strikingly similar to those of Piper(1) for the electric change, and one can only conclude that they are due to the same cause, viz. to a tetanus provided by the nervous system. In that case the incomplete nature of the tetanus is striking evidence that the ordinary voluntary contraction is due to

nothing but a tetanus, not to some kind of continuous stimulus produced by some mysterious process unknown. One striking result emerges from these results, as from those of Piper. If the various nerve cells innervating a muscle were not working at the same frequency and in phase with one another, the discordant phases of all the separate stimuli would average out and make the resulting contraction or the resulting electric change appear to be a continuous one, not discontinuous as observed. The fact that the oscillations are of so distinctly a harmonic nature suggests that

(*A*), (*B*), and (*C*), glycerine tambour, (*D*) plain tambour, with very tight membrane. The oscillations have been marked beneath in ink on the record, to assist in counting and to show the reader what have been taken as oscillations. In (*C*) the glycerine tambour was struck sharply several times to show its natural period of vibration which is considerably less than that of the vibrations shown in (*A*) and (*B*): the plain tambour has a still more rapid period. Time in units of 0·0164 second, with a gap every 12 units.

all the separate stimuli from the separate cells in the nervous system are of the same frequency and in phase with one another, a matter which would appear to be of fundamental interest in the behaviour of the nerve cells making up a "centre" in the nervous system, and one worthy of specific investigation.

In one subject (A. V. H.) the vibrations are regularly about 48 per second, while in others they are rather slower, viz. about 40. It is hoped to continue a study of these oscillations in health, fatigue and disease,

and in reference to various kinds of reflex or voluntary response. They are easy to record, much easier than the electric change, and they seem to exhibit a very fundamental property of the activity of the neuro-muscular system.

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(1) Piper. Elektrophysiologie menschlicher Muskeln, p. 84 etc. Springer, Berlin, 1912.

On the relation of K in Hill's equation to the carbonic acid pressure in the blood. By G. S. ADAIR.

L. J. Henderson showed that for a series of oxygen dissociation curves of Barcroft's blood exposed to different pressures of carbonic acid, the carbonic acid pressure bore a linear relation to the reciprocal of K in Hill's equation. During the year 1918, I had the opportunity of testing this relationship on the bloods of a large number of soldiers and munition workers suffering from dyspnœa, and more recently on ten additional normal persons. The law may be expressed as follows

$$1/K = au + b,$$

where u is the CO_2 pressure and a and b are constants.

$1/K$ is a measure of the readiness with which blood parts with its oxygen.

The following example will show the deviations from the straight line

CO_2 pressure	0·6	10·9	25·3	38·7	57·6	68·4
$1/K$ observed	330	1560	3350	4450	5850	8050
$1/K$ calculated	425	1570	3170	4690	6100	7960

The correspondence is more close if the observed values of K are not obtained from single points as above, but from whole curves as in the case of Barcroft. The data for the carboxy-hæmoglobin curves for the blood of Douglas are given below:

CO_2 pressure	0	19	42	79
$1/K$ observed	·0009	·0032	·0070	·0120
$1/K$ calculated	·0010	·0035	·0069	·0119

The range of normals (see Fig. 1).

The normal persons recently observed give values of a and b between the following limits:

(a) 92–68, (b) 400–700.

The dyspnœic cases studied gave in most cases a much higher value of a (see Fig. 1).

Range of (a) 75–187, (b) 320–1000.

The applicability of the law to the case of other acids.

There is no reason at present to suppose that acids other than carbonic conform to the law. The few observations available show that lactic acid behaves in quite a different manner.

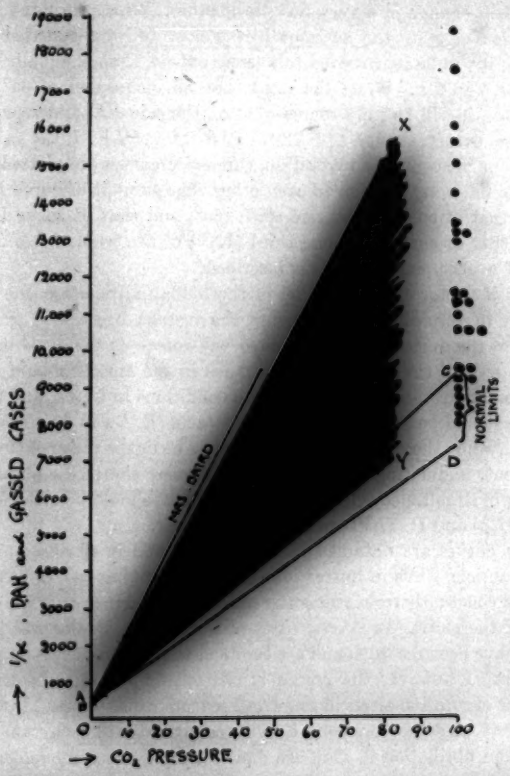


Fig. 1. Ordinate $= 1/K$. Abscissa $= CO_2$ pressure. The area $ABCD$ encloses lines given by ten consecutive normal individuals, and $ABXY$ encloses lines of 25 soldiers and munition workers suffering from dyspnœa. The points above the 100 mm. line show where these latter would run out if continued and therefore give an idea of their distribution.

Evidence of circus movement in the human auricle. By T. Lewis, A. N. Drury and C. C. Iliescu[1]. (*Preliminary notice.*)

If a given E.M.F. represented by the tangential arrows in the accompanying figure, is revolved through 360°, then, as it revolves, its representation upon the three sides of a surrounding equilateral triangle (*RLF*) will change in a very definite manner. When the E.M.F. has an inclination of 0° to the horizontal, it will be fully represented on the side *RL*, it will be represented to a lesser extent, though equally, on the sides *RF* and *LF*. When the E.M.F. has an inclination of 60° to the horizontal, it will be fully represented on the side *RF*, and represented to a lesser extent, though equally, on sides *RL* and *LF*. If the values are plotted for the complete revolution, three *sine* curves are plotted, which have the following relation to each other. The summit of curve III lags behind that of curve II by one sixth (60°) and that of curve III lags behind that of curve I by one third (120°) of the whole cycle. Thus a series of overlapping summits is inscribed.

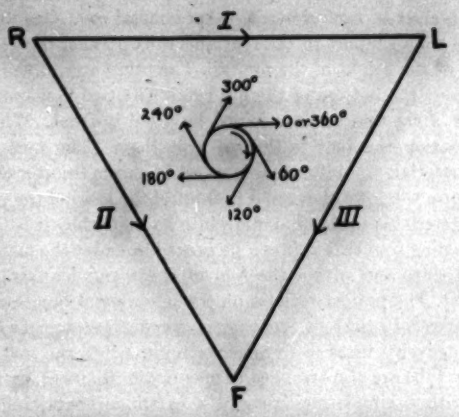
Now if flutter and fibrillation of the human auricle are due fundamentally to simple circus movements, the average direction in which the muscle of the auricle becomes excited will rotate as the cycle proceeds and the axis of the electrical disturbance in the auricle should show a corresponding rotation. If such be the conditions in flutter and fibrillation, we should be able to place three contacts (*R*, *L* and *F*) on the chest wall and, when these are so placed that they lie in the plane of the circus movement, undulating and overlapping curves[2] should be obtained in any pair of simultaneous leads, and the overlaps should be greatest when leads I (*RL*) and III (*LF*) are used.

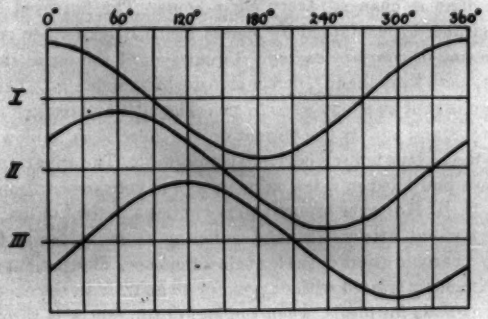
Such curves are obtained both in flutter and in fibrillation of the human auricle. Thus in flutter (one case) overlapping curves have been obtained constantly from triangular contacts placed in or near the sagittal plane of the chest. In several cases of fibrillation, similar overlapping curves have been recorded and are confined to leads from certain planes; in fibrillation, however, the overlap is only seen in phases, indicating that the plane of rotation alters in some degree from time to time. It appears to us that these observations if fully confirmed will *prove* that both flutter and fibrillation in man are due to simple circus movements. It

[1] Working on behalf of the Medical Research Council.

[2] As the auricle is not a flat and circular sheet of muscle, and as the movement is not necessarily circular but may be irregularly elliptical, pure *sine* curves are not to be expected.

will be evident that by means of direct chest leads we may be able to detect not only the plane of the main circus movement, but also the direction (clockwise or anti-clockwise as the case may be) in which the wave rotates in this plane.





A simple oxygen bed tent and its use to a case of œdema and chronic ulcer of the leg. By LEONARD HILL.

For the convenient administration of oxygen to patients, who will not, or cannot, put up with the use of a mask, a tent oxygen chamber has been contrived so as to enclose the upper part of the patient when in bed. A light metal bar hooks on to the top rail of the bedstead; to this is attached on each side a V-shaped metal rod. One arm of the V forms the wing support to the tent and runs parallel to the side of the bedstead at the height of the top rail. The other arm of the V supports itself against the bottom of the head rail. The two V-shaped rods form the wings of the tent and are joined by a rod in front. When the rods are in position the tent is thrown over them. The tent is made of balloon material, non-inflammable celluloid sheets being inserted into it so as to form windows above and in front of the head of the patient. The balloon material is made of ample width so that when it is thrown over the supporting framework, it can be tucked in under the mattress at the back and sides and within the fold of sheet and blanket in front of the patient. The patient reclines on the pillows and breathes the atmosphere enclosed by the tent. An oxygen cylinder is arranged with reducing valve to deliver 5 litres of oxygen per minute into the tent[1]. An inlet and outlet tube are also arranged to open within the tent, and connected outside with a circulating fan and a cartridge containing soda-lime. The soda-lime is changed every eight hours. The fan used is of the vacuum cleaner type adapted by Messrs Keith Blackman to run either on alternating or constant current. Circulation of the air in the tent is kept up so as to keep the CO_2 percentage down to about 0·5. The oxygen percentage is kept at about 35 to 37 p.c. The inlet and oxygen tubes are tied together, and laid by the patient on the sheet so as to blow over his face. He thus keeps himself cool and comfortable. The apparatus can be taken down and fixed in a few minutes and is very easy to run.

Mr J. E. H. Roberts brought to my notice a patient of his, a soldier with his femoral artery tied and suffering from coldness, blueness, œdema and chronic ulcers of the leg below the knee. This patient has been thus continuously treated with oxygen for 48 hours with the exception of half hour periods for meals, when the flap of the tent was thrown back and the administration of oxygen temporarily discontinued. The oxygen quickly made the feet and leg remarkably warm and of excellent colour, removed the œdema, cleaned up the ulcers and started healing these in

[1] The cost of oxygen at a penny a cubic foot is about ten-pence an hour.

a way which appeared very striking to the medical officers and nurses familiar with the chronic intractable condition of this patient's leg and others similarly affected. On stopping the continuous administration and substituting inhalations for 10 minutes per hour from a Haldane mask (none when asleep) the beneficial effect markedly lessened. A second case treated for 4 days with oxygen gave similar beneficial results.

The theory which led to the oxygen treatment is as follows: œdema is not due to a transudation from the capillaries produced by capillary pressure, but to increased osmotic pressure of ill-nourished tissue cells. In the patient there was deficient anastomosis and circulation; no pulsation in the posterior tibial or dorsalis pedis arteries could be felt; the colour of the skin was bluish. The œdema and blue colour became greatly enhanced when the patient hung his leg down. Want of oxygen was an obvious symptom, and this want produced degradation products in the tissues which caused their swelling by osmosis. The swelling confined by the skin and aponeurosis pressed upon the capillaries and veins and so raised the capillary-venous pressure towards the arterial pressure. It took a jet of water 200 cm. high to drive the blood out of a small area of the skin of the patient's foot when this was blue and swollen, while a jet of 50 cm. high easily blanched the skin in the normal foot. By saturating the blood with oxygen it was conceived that the tissue cells would recover as the waste substances become oxidised, the local circulation would in consequence improve, the œdema disappear, and healing of the ulcers take place.

The results abundantly confirmed the conception and suggest that oxygen inhalation may contribute greatly to the healing of chronic ulcers and relief of œdema due to circulatory defect.

I am indebted to Mr R. H. Davis and Mr Rosling of Messrs Siebe Gorman Ltd. for help in constructing the oxygen bed tent, to Mr J. E. H. Roberts for allowing me to treat the patient with oxygen and his interest in this treatment, and to Dr Argyle Campbell and Mr A. Webster, for help in carrying on the treatment of the first case by night. The treatment of the second case was carried out by the nurses successfully.

Brownian movement and fibril formation. By J. O. Wakelin Barratt.

Particles of sufficiently small dimensions suspended in liquid exhibit oscillatory motion, usually termed Brownian movement and now recognised to be indicative of molecular motion. So long as the particles show no tendency to adhere the system remains unchanged. Should a sus-

pended particle exhibit this tendency, however, it would sooner or later become attached to a surface with which it might happen to collide and if, in addition, the material of which the particle was composed were highly viscous it would upon recoil become drawn out forming a connecting thread extending between the surface with which it had collided and the main mass of the particle. If a collision now took place with a second surface the thread would become attached at each end. It is thus obvious that, as a result of oscillatory movement, suspended particles, if composed of viscous material and possessing the property of adhering to a surface, may be expected to undergo processes involving change of form and, it may be, resulting in the production of more or less elaborate structural effects. One such process—among others—is fibril formation. If it is assumed that the fibrils exhibited by gels (*e.g.* of soap, fibrin, barium malonate) arise in this way, it becomes possible to form some idea of the size of the particles giving origin to fibrils and to explain the effect on fibril formation of dilution of the hydrosol in which the fibrils appear. Thus in the case of fibrinogen the rapidity with which fibrils are produced indicates that their particles of origin are subject to much greater translatory movement than is shown by the smallest particles visible under ultramicroscopic illumination (about $0{\cdot}2\mu$ in apparent diameter): the particles of origin must therefore be of amicroscopic dimensions. The fibrils first formed are similarly inferred to be also amicroscopic: they subsequently increase in thickness, however, at the expense of the continuous phase of the fibrinogen solution employed and in this way enter into the range of ultramicroscopic (and later of microscopic) visibility. When no such "growth" takes place the fibrils constituting the gel remain permanently amicroscopic and the gel is apparently structureless under ultramicroscopic illumination. So extreme is the degree of fineness of fibril and closeness of mesh in gels of nitrocotton, used as filters, that SO_4^{--} ions may be arrested, though the passage of Cl^- and Na^+ ions is permitted. When a hydrosol of fibrinogen is diluted the number of particles forming the disperse phase and giving origin to fibrils is correspondingly diminished. If at the same time the concentration of the continuous phase is sufficiently lowered, the fibrils formed do not become visible though "setting" may still take place, disappearing, however, on still further dilution.

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